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Transient reversion of ras oncogene-induced cell transformation by antibodies specific for amino acid 12 of ras protein. .

Nature, (Apr 18-24 1985) Vol. 314, No. 6012, pp. 639-42. Journal code: 0410462. ISSN: 0028-0836.; English

virus (TGGTACAAAATGTTCT) or *MT2* transcription[8,14], or in metal ion induction[8] of *MT2* ($^{TG}_{GT}$CGCCCGGC$^{C}_{C}$). It is also more extensive than the consensus sequence for transcriptional regulation of heat shock genes (GTNGAANNTTCNAG) which has been shown to function in mammalian cells, *Xenopus* oocytes and *Drosophila*[18–20]. General regulation of yeast amino-acid biosynthesis also depends on a short regulatory sequence (A^{A}_{T}GTGACTC)[21,22]. The sequences involved in regulation of transcription by glucocorticoids, metal ions or heat shock are conserved among different species. We have found no homology between the consensus sequence shown in Fig. 4 and sequences 5′ of mouse *H-2* genes, but the mouse sequences available in the NIH and EMBL databases do not extend far beyond the 5′ start sites of the mRNAs.

Experiments are now under way to test directly the role of the consensus sequence in transcriptional regulation by interferon. If this sequence is involved, it may be long enough to be used to isolate genes directly from phage or cosmid libraries by hybridization, and would help in locating their regulatory regions. Both consensus and naturally occurring sequences might then be used to isolate the proteins responsible for interferon transcriptional regulation.

We thank Warren Liao for helpful discussions, James Darnell for communicating results before publication and Peter Gunning and Lawrence Kedes for the β-actin cDNA clone. This work was supported by PHS grant CA30124 from the National Cancer Institute. R.L.F. is a trainee of the NIH Medical Scientist Training Program.

Received 12 December 1984; accepted 23 January 1985.

1. Chebath, J., Merlin, G., Metz, R., Benech, P. & Revel, M. *Nucleic Acids Res.* **11**, 1213–1226 (1983).
2. Merlin, G., Chebath, J., Benech, P., Metz, R. & Revel, M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4904–4908 (1983).
3. Friedman, R. L., Manly, S. P., McMahon, M., Kerr, I. M. & Stark, G. R. *Cell* **38**, 745–755 (1984).
4. Larner, A. C. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 6733–6737 (1984).
5. Rosa, F. *et al. EMBO J.* **2**, 1583–1589 (1983).
6. Karin, M., Andersen, R. D., Slater, E., Smith, K. & Herschman, H. R. *Nature* **286**, 295–297 (1980).
7. Karin, M., Anders·n, R. D. & Herschman, H. R. *Eur. J. Biochem.* **118**, 527–531 (1981).
8. Karin, M. *et al. Nature* **308**, 513–519 (1984).
9. Sood, A. K., Pereira, D. & Weissman, S. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 616–620 (1981).
10. Gunning, P. *et al. Molec. cell. Biol.* **3**, 787–795 (1983).
11. Ringold, G. M., Dieckmann, B., Vannice, J. L., Trahey, M. & McCormick, F. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3964–3968 (1984).
12. Yoshie, O. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 649–653 (1984).
13. Vannice, J. L., Taylor, J. M. & Ringold, G. M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4241–4245 (1984).
14. Richards, R. I., Heguy, A. & Karin, M. *Cell* **37**, 263–272 (1984).
15. Strachan, T., Sodoyer, R., Damotte, M. & Jordan, B. R. *EMBO J.* **3**, 887–894 (1984).
16. Malissen, M., Malissen, B. & Jordan, B. R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 893–897 (1982).
17. Das, H. K., Lawrence, S. K. & Weissman, S. M. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3543–3547 (1983).
18. Bienz, M. & Pelham, H. R. B. *EMBO J.* **1**, 1583–1588 (1982).
19. Pelham, H. R. B. *Cell* **30**, 517–528 (1982).
20. Pelham, H. R. B. & Bienz, M. *EMBO J.* **1**, 1473–1477 (1982).
21. Donahue, T. F., Daves, R. S., Lucchini, G. & Fink, G. R. *Cell* **32**, 89–98 (1983).
22. Lucchini, G., Hinnebusch, A. G., Chen, C. & Fink, G. R. *Molec. cell. Biol.* **4**, 1326–1333 (1984).
23. Allen, G., Fantes, K. H., Burke, D. C. & Morser, J. *J. gen. Virol.* **63**, 207–212 (1982).
24. Karin, M. & Richards, R. I. *Nature* **299**, 797–802 (1982).
25. Wilbur, W. J. & Lipman, D. J. *Proc. natn. Acad. Sci. U.S.A.* **80**, 726–730 (1983).

Transient reversion of *ras* oncogene-induced cell transformation by antibodies specific for amino acid 12 of *ras* protein

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The proteins encoded by the *ras* oncogene are thought to trigger expression of the transformed phenotype in some types of cancer cells. In human cells, the *ras* protein family consists of several members including normal (proto-oncogene) and mutant (oncogene) forms[1–3]. In general, the proto-oncogene forms are thought to be involved in the normal growth control of cells, while the mutant forms (which apparently result from somatic mutation of the normal *ras* genes) appear to be responsible, in part, for the loss of normal growth control. On microinjection into living normal cells, the purified *ras* oncogene protein (p21) induces a characteristic loss of growth control in cells within several hours[4,5]. The mutant forms of the different *ras* proteins typically contain a single amino-acid change, usually at position 12 or less frequently at position 61 (ref. 6). Here we report that microinjection of antibodies specific for amino acid 12 of the oncogenic v-Ki-*ras* protein into cells transformed by this protein causes a transient reversion of the cells to a normal phenotype. The fact that this antibody inhibits binding of GTP to the v-Ki-*ras* protein supports the notion that GTP binding is essential to the transforming function of this oncogene product.

The role of *ras* proteins in cellular metabolism is not yet understood, but it is known that they are localized on the inner surface of the plasma membrane and bind GTP and GDP[7,8]. Recent analysis of the normal and mutant forms of the human Ha-*ras* proteins purified from bacterial expression systems[9,10] or present in the bacterial lysates after expression[11] has shown that *ras* proteins are able to hydrolyse GTP to GDP. It has been

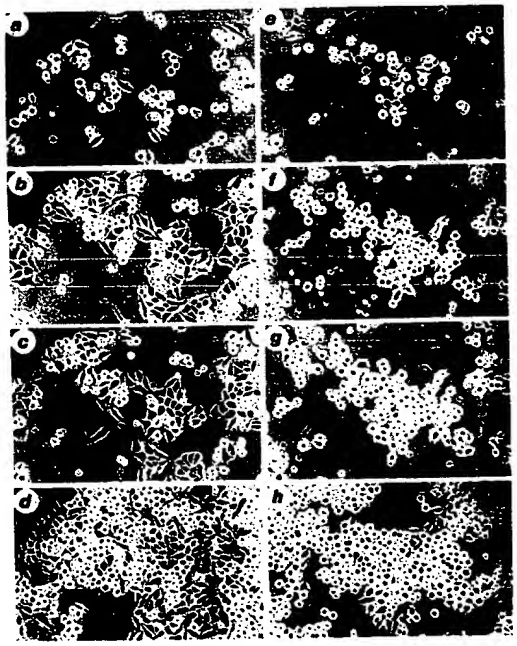

Fig. 1 Morphology of Ki-NRK cells after microinjection of anti-p21-Ser and control IgG. Approximately 35 Ki-NRK cells (growing in 10% fetal calf serum in Dulbecco's modified Eagle's medium) in each of the two test areas (marked by ink circles under the dish) were microinjected with anti-p21-Ser (*a–d*) or non-immune IgG (*e–h*) as described elsewhere[18]. Phase-contrast photomicrographs were taken of the two areas 30 min (*a*, *e*), 24 h (*b*, *f*), 36 h (*c*, *g*) and 48 h (*d*, *h*) after injection. Note that the cells injected with anti-p21-Ser show a normal flattened morphology for some time and grow at a slower rate than either the surrounding uninjected cells or the cells injected with the control IgG. ×4.

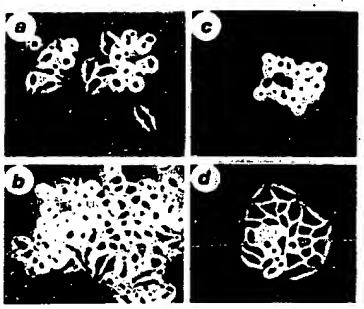

Fig. 2 Slower growth rate of Ki-NRK cells after microinjection of anti-p21-Ser antibody. Two areas of sparsely plated Ki-NRK cells growing on a dish (see Fig. 1 legend) were injected with either control IgG (*a*) or anti-p21-Ser IgG (*c*). The two areas were photographed immediately after injection (*a* and *c*) and 24 h later (*b* and *d*, respectively). Note the different rates of growth between the two areas of cells.

suggested that *ras* proteins function in a manner analogous to the 'G' regulatory protein of the adenylate cyclase system[4,9-13], which is also localized in the plasma membrane; this model is particularly attractive because G proteins activate adenylate cyclase through a GTP-dependent reaction, and inactivate the enzyme through hydrolysis of bound GTP to GDP and P_i. Position 12 mutants of human Ha-*ras* protein show a 10-fold reduction in rate of GTP hydrolysis[9-11], and may therefore represent abnormally activated regulatory proteins.

To obtain a biochemical probe capable of distinguishing between the normal and oncogenic forms of the *ras* proteins, antibodies were raised against *ras* (p21)-related peptides that differed at position 12. One antibody, designated anti-p21-Ser, was affinity-purified from the serum of rabbits immunized with the peptide ^{6}Lys-Leu-Val-Val-Gly-Ala-Ser-Gly-Val-Gly-Lys Cys-^{18}Ser (Ser at position 12) coupled covalently to carrier protein (R.C., G.W., D. Nitecki and F. McC., unpublished). Anti-p21-Ser was found to bind the v-Ki-*ras* protein (serine at position 12) but neither the v-Ha-*ras* protein (arginine at position 12) nor the *ras* protein containing glycine (normal) at position 12. In addition, biochemical studies showed that this antibody blocked both the GTP-dependent autophosphorylation and GTP-binding activities of the v-Ki-*ras* protein, suggesting that the antibody and GTP bind to the same region of the protein.

Given this high degree of specificity of the affinity-purified antibody preparation for the oncogenic v-Ki-*ras* protein, as well as the ability of the antibody to inhibit the GTP binding activity of the v-Ki-*ras* protein, it seemed possible that introduction of this antibody preparation into living, transformed cells might block the biochemical action of the *ras* oncogene protein *in vivo*, without affecting the function of the proto-oncogene product. Using microneedle injection procedures, $\sim 5 \times 10^5$ molecules of purified IgG (either anti-p21-Ser or non-immune serum) were injected into the cytoplasm of NRK cells transformed by the v-Ki-*ras* oncogene (called Ki-NRK cells); this represents a 5–10-fold excess of antibody over the estimated amount of *ras* protein in these cells[4]. Within 15 h and persisting for 48 h after injection of the anti-p21-Ser antibodies, the Ki-NRK cells showed a flattened, normal morphology (Fig. 1*a–d*); after 48 h, the cells reverted to their rounded morphology. In contrast, cells on the same dish which had been injected with control IgG showed no obvious change in morphology (Fig. 1*e–h*). (Control IgGs included goat anti-mouse IgG and rabbit non-immune IgG.) Inspection of the photographs taken for these experiments indicated that in addition to the reversion of the transformed phenotype, the flattened cells appeared to grow at a slower rate than either cells injected with control IgG or surrounding uninjected cells; this is evident from Fig. 2, where cells injected with control IgG have tripled in number over 24 h post-injection while cells injected with the anti-p21-Ser IgG have only doubled in number over the same time period. Based on data from the genetic analysis of the yeast *ras* genes which indicated that complete elimination of *ras* gene expression yielded non-viable cells[14,15], we might predict that antibodies that block the function of both the normal and oncogenic forms of the *ras* proteins would kill or prevent growth of microinjected transformed mammalian cells. Figures 1 and 2 show, however, that the cells injected with anti-p21-Ser are indeed able to grow, a result in agreement with biochemical experiments which showed that this antibody binds only to the oncogenic form of the *ras* protein with serine at position 12 (R.C., G.W., N.A., D. Nitecki and F.McC., unpublished).

By using immunofluorescence microscopy, we were able to determine exactly which cells had been injected with the two antibody preparations. Those cells that had been injected with

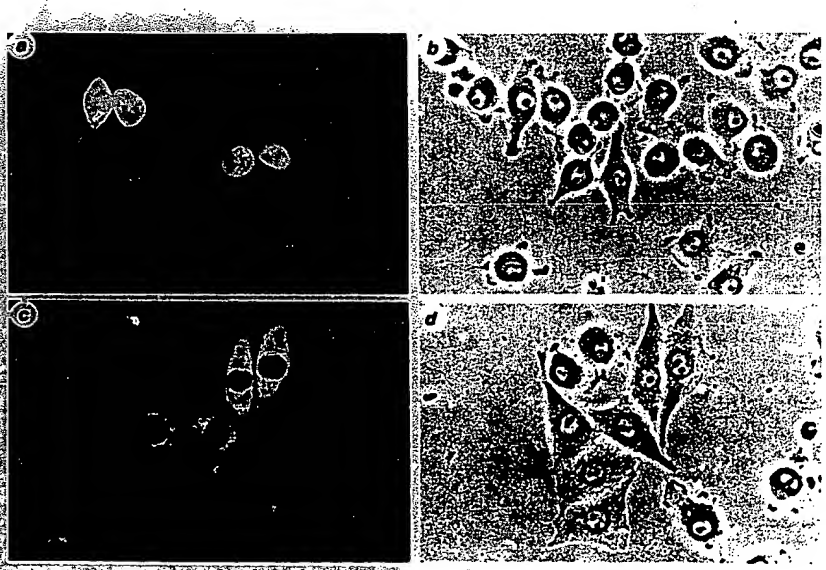

Fig. 3 Correspondence of the flattened appearance of Ki-NRK cells with microinjection of anti-p21-Ser antibodies. Ki-NRK cells (growing on glass coverslips) were injected with non-immune IgG (*a*, *b*) or anti-p21-Ser (*c*, *d*) and fixed (absolute methanol, −20 °C, 5 min) 24 h after injection. The cells were treated with fluorescein isothiocyanate-labelled goat anti-rabbit IgG (Cappel Laboratories), which stained only the injected cells. The fields of cells were photographed by epifluorescence (*a*, *c*) and phase-contrast (*b*, *d*) microscopy with a Zeiss Photomicroscope III as described elsewhere[18]. *a*, *b* Represent the same field of cells injected with control IgG and *c* and *d* the same field of cells injected with anti-p21-Ser.

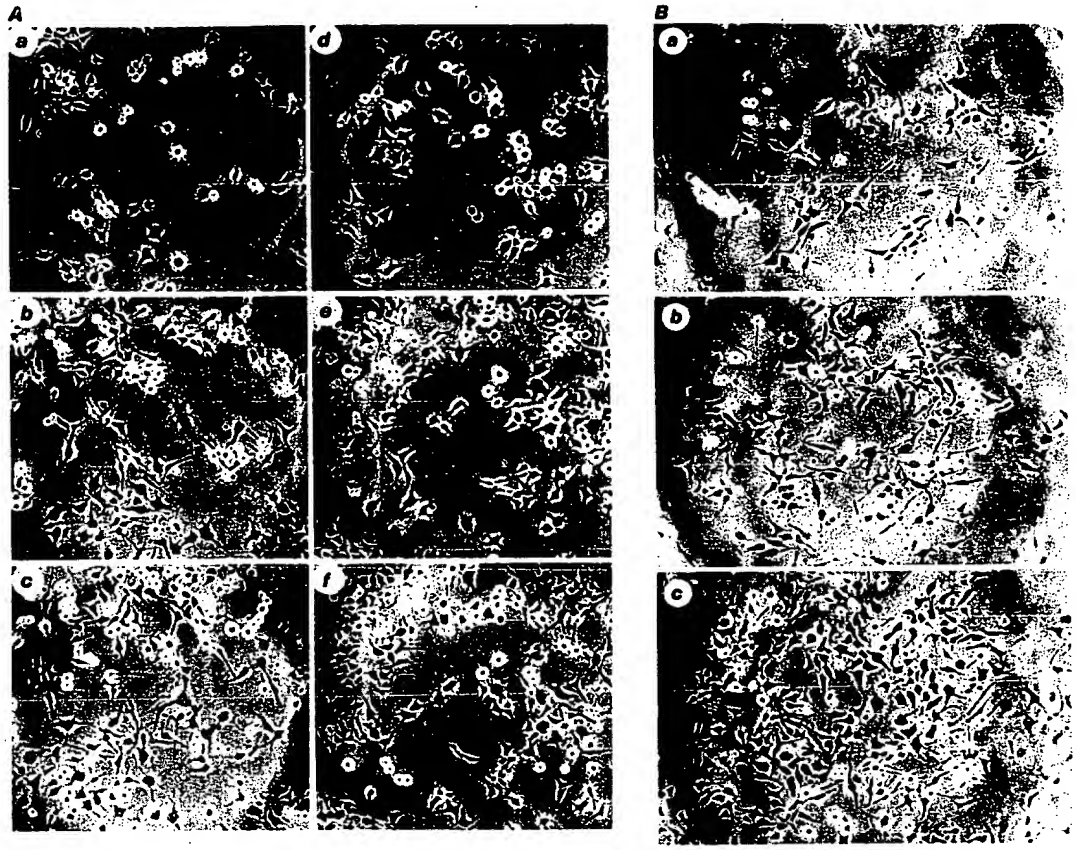

Fig. 4 Morphology of Ha-NRK cells after microinjection of: *A*, anti-p21-Ser and control IgG; and *B*, monoclonal antibody Y-238. *A*, Approximately 25 Ha-NRK cells in defined areas (marked by ink circles on the bottom of the dish) were injected with anti-p21-Ser (*a–c*) or non-immune IgG (*d–f*). The areas were photographed 30 min (*a, b*), 24 h (*c, d*) and 36 h (*e, f*) post-injection. No change in morphology or growth rate was observed for either antibody. *B*, Approximately 30 Ha-NRK cells were injected with monoclonal antibody Y-238 (ref. 16) and photographed 30 min (*a*), 24 h (*b*), and 36 h (*c*) after injection. Note the lack of effect of this monoclonal antibody on either the morphology or the growth rate of the cells.

anti-p21-Ser had the morphologically normal, flattened appearance (Fig. 3*c, d*) whereas cells injected with control IgG retained their rounded, transformed appearance (Fig. 3*a, b*).

To determine the specificity of the *in vivo* effect of the anti-p21-Ser preparation in terms of cells transformed by other oncogenic *ras* proteins, the anti-p21-Ser and control IgGs were injected (5×10^5 molecules per cell) into Ha-NRK cells (that is, NRK cells transformed by the v-Ha-*ras* oncogene protein, which has an arginine at position 12). Photomicroscopy indicated that neither antibody had an effect on these cells (Fig. 4*A*); this result is consistent with the observation that, *in vitro*, the anti-p21-Ser preparation does not react with the v-Ha-*ras* protein (R.C., G.W., N.A., D. Nitecki and F.McC., unpublished).

To determine whether the ability of anti-p21-Ser to induce reversion to normal phenotype is a general property of anti-p21 antibodies, one other antibody was tested by this assay. Furth and co-workers have produced several rat-derived monoclonal antibodies against the v-Ha-*ras* protein[16], including one that is relatively specific for the Ha-*ras* proteins (called Y-238); this antibody does not block GTP binding activity, does not discriminate between normal and mutant forms of the *ras* protein and probably binds to a site in the Ha-*ras* protein that is not influenced by amino acid 12 of the protein. Y-238 was microinjected into Ha-NRK cells (1×10^6 molecules per cell), and the cells were examined by photomicroscopy for 48 h. Neither the Y-238 monoclonal antibody nor a control IgG preparation had any effect on the morphology or growth rate of the cells (Fig. 4*B*); similar results were obtained using Y-259 anti-p21 antibody. Thus, the antibody either binds to a non-essential site on the *ras* protein or does not bind at all to the *ras* protein in these cells.

These results, together with the ability of anti-p21-Ser to block GTP binding by p21 *in vitro* (R.C., G.W., N.A., D. Nitecki and F.McC., unpublished), are consistent with the idea that the interaction of p21 with GTP is crucial to the transforming function of the *ras* gene. However, further studies are required to examine the possibility that the antibody disrupts other important activities of p21 such as membrane localization or interactions with cellular proteins. Whatever mechanism is responsible for the observed effects of the microinjected anti-p21-Ser on cell behaviour, the data presented here indicate that antibodies capable of inactivating oncogene but not proto-oncogene products can block the expression of the transformed phenotype in living cells. Gallick *et al.*, using antibodies directed against the $p37^{mos}$ oncogene product of the Moloney murine sarcoma virus, reached a similar conclusion; microinjection of the antibodies yielded at least a different morphology in transformed cells[17].

When antibodies specific for other single mutations in the *ras* protein family (N.A. *et al.*, in preparation) become available, we will be able to extend this approach to inhibition of the function of the other forms of the oncogenic *ras* proteins in living cell and biochemical systems. Such experiments, coupled with studies in which the human *ras* oncogene protein is injected into normal cells, resulting in transient proliferation[4,5], provide new ways of examining the physiology and cell biochemistry of the transformation process.

We thank J. D. Watson for his support, and Drs D. Bar-Sagi, N. Sullivan, W. Welch and M. Wigler for helpful discussions. We thank Dr Mark Furth of the Memorial Sloan-Kettering Cancer Center for his gift of monoclonal antibodies against the *ras* protein (Y-238 and Y-259), and acknowledge the technical assistance of N. Kronenberg, C. Hallaran, P. Renna and M. Szadkowski. This work was supported in part by NIH grant GM28277 (to J.R.F.).

Received 6 November 1984; accepted 4 February 1985.

1. Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* 79, 3637–3640 (1982).
2. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* 297, 474–478 (1982).
3. Shimizu, K. *et al. Proc. natn. Acad. Sci. U.S.A.* 80, 2112–2116 (1983).
4. Feramisco, J. R., Kamata, T., Gross, M., Rosenberg, M. & Sweet, R. W. *Cell* 38, 109–117 (1984).
5. Stacey, D. & Kung, H. *Nature* 310, 508–511 (1984).
6. Marshall, C. J. & Rigby, P. W. J. *Cancer Surv.* 3, 183–214 (1984).
7. Willingham, M. C., Pastan, I., Shih, T. Y. & Scolnick, E. M. *Cell* 19, 1005–1014 (1980).
8. Shih, T. Y., Papageorge, A. G., Stoker, P. E., Weeks, M. O. & Scolnick, E. M. *Nature* 287, 686–691 (1980).
9. Sweet, R. W. *et al. Nature* 311, 273–275 (1984).
10. Gibbs, J. B., Sigal, I. S., Poe, M. & Scolnick, E. M. *Proc. natn. Acad. Sci. U.S.A.* 81, 5704–5708 (1984).
11. McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. *Nature* 310, 644–649 (1984).
12. Gilman, A. G. *Cell* 36, 577–579 (1984).
13. Kamata, T. & Feramisco, J. R. *Cancer Cells* 1, 11–16 (1984).
14. Tatchell, K., Chaleff, D., Defeo-Jones, D. & Scolnick, E. *Nature* 309, 523–527 (1984).
15. Kataoka, T. *et al. Cell* 37, 437–455 (1984).
16. Furth, M. E., Davis, L. J., Fleurdelys, B. & Scolnick, E. M. *J. Virol.* 43, 294–304 (1982).
17. Gallick, G. E., Brown, D. B., Murphy, E. C. Jr & Arlinghaus, R. B. in *Modern Approaches to Vaccines* (eds Chanock, R. M. & Lerner, R. A.) 407–413 (Cold Spring Harbor Laboratory, New York).
18. Wang, K., Feramisco, J. R. & Ash, J. F. *Meth. Enzym.* 85, 514–562 (1982).

Production of 'hybrid' antibiotics by genetic engineering

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The recent development of molecular cloning systems in *Streptomyces*[1–4] has made possible the isolation of biosynthetic genes for some of the many antibiotics produced by members of this important genus of bacteria[5–10]. Such clones can now be used to test the idea that novel antibiotics could arise through the transfer of biosynthetic genes between streptomycetes producing different antibiotics[11]. The likelihood of a 'hybrid' compound being produced must depend on the substrate specificities of the biosynthetic enzymes, about which little is known. In attempts to demonstrate hybrid antibiotic production, we therefore began with strains producing different members of the same chemical class of compounds in order to maximize the chance of success. Here we report the production of novel compounds by gene transfer between strains producing the isochromanequinone antibiotics actinorhodin[12], granaticin[13] and medermycin[14]. These experiments were made possible by the recent cloning of the whole set of genes for the biosynthetic pathway of actinorhodin from *Streptomyces coelicolor* A3(2) (ref. 8). We believe that this represents the first report of the production of hybrid antibiotics by genetic engineering.

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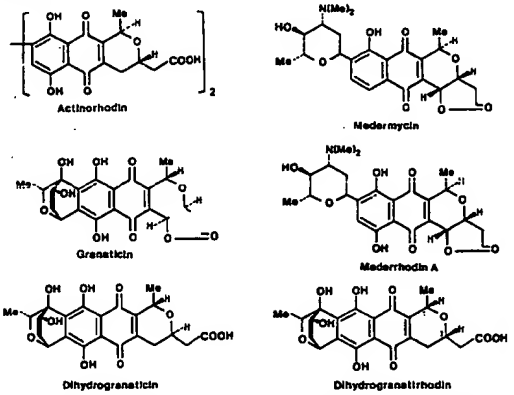

Fig. 1 Structures of the isochromanequinone antibiotics. The absolute stereochemistry of dihydrogranatirhodin is tentative (see text).

Plasmid pIJ2303 (ref. 8) consists of the low-copy-number vector plasmid pIJ922, derived from SCP2* (ref. 15), with 32.5 kilobases (kb) of *S. coelicolor* A3(2) DNA inserted at its unique *Bam*HI site[8]. That this inserted DNA carries the complete cluster of genes (*act*) for the biosynthesis of actinorhodin (Fig. 1) from simple primary metabolites (probably acetate) is shown by complementation of all available classes of *S. coelicolor act* mutants by pIJ2303 and by the production of actinorhodin by several species of *Streptomyces* (including *S. parvulus* ATCC 12434, *S. peucetius* ATCC 29050, *S. tanashiensis* KA-415 and *S. glaucescens* ETH 22794) carrying pIJ2303 (F.M., H.M.K. and D.A.H., unpublished).

In an analysis of the organization and expression of the *act* genes, segments of the total *act* DNA of pIJ2303 have recently been inserted (F.M., unpublished) into the *Bam*HI site of pIJ922 or into the *Bam*HI, *Bgl*II or *Pst*I sites of pIJ940 (a second low-copy-number vector derived from SCP2*; ref. 15). Some of these subclones, carrying different transcription units of the *act* region, were introduced by transformation into *Streptomyces* sp. AM-7161 (the producer of medermycin), *Streptomyces violaceoruber* Tü22 (the producer of granaticin), and a mutant (B1140) of Tü22 blocked in granaticin synthesis, by selecting for the thiostrepton-resistance marker of the vectors. The efficiency of transformation of these cultures, using standard conditions developed for *Streptomyces lividans* and *S. coelicolor*[16], by DNA of the clones (isolated from *S. coelicolor* A3(2) derivatives) was only 1–10 per μg of plasmid DNA, compared with 10^6–10^7 in *S. coelicolor* A3(2) (ref. 8). These reduced frequencies may reflect the operation of restriction systems, as well as the fact that no attempt was made to optimize transformation conditions for these strains. Nevertheless, transformants were obtained in all of the combinations attempted (Table 1).

Actinorhodin, granaticin and medermycin are all acid–base indicators which confer characteristic colours on the cultures producing them: red↔blue for actinorhodin; red↔purple for granaticin; and yellow↔brown for medermycin. It was apparent from the colours of AM-7161 and B1140 carrying pIJ2303 that the *S. coelicolor act* genes were being expressed in the recipient strains, as blue pigments were present in alkaline conditions. More strikingly, the colours of the cultures produced by introducing certain of the partial *act* clones into the medermycin producer (AM-7161) indicated that one or more novel compounds were present. Notable was the bright purple colour, at

Originally published In Press as doi:10.1074/jbc.M200328200 on April 8, 2002

J. Biol. Chem., Vol. 277, Issue 25, 23065-23075, June 21, 2002

c-Raf/MEK/ERK Pathway Controls Protein Kinase C-mediated p70S6K Activation in Adult Cardiac Muscle Cells*

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Received for publication, March 14, 2002, and in revised form, April 5, 2002

ABSTRACT

p70S6 kinase (S6K1) plays a pivotal role in hypertrophic cardiac growth via ribosomal biogenesis. In pressure-overloaded myocardium, we show S6K1 activation accompanied by activation of protein kinase C (PKC), c-Raf, and mitogen-activated protein kinases (MAPKs). To explore the importance of the c-Raf/MAPK kinase (MEK)/MAPK pathway, we stimulated adult feline cardiomyocytes with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), insulin, or forskolin to activate PKC, phosphatidylinositol-3-OH kinase, or protein kinase A (PKA), respectively. These treatments resulted in S6K1 activation with Thr-389 phosphorylation as well as mammalian target of rapamycin (mTOR) and S6 protein phosphorylation. Thr-421/Ser-424 phosphorylation of S6K1 was observed predominantly in TPA-treated cells. Dominant negative c-Raf expression or a MEK1/2 inhibitor (U0126) treatment showed a profound blocking effect only on the TPA-stimulated phosphorylation of S6K1 and mTOR.

Whereas p38 MAPK inhibitors exhibited only partial effect, MAPK-phosphatase-3 expression significantly blocked the TPA-stimulated S6K1 and mTOR phosphorylation. Inhibition of mTOR with rapamycin blocked the Thr-389 but not the Thr-421/Ser-424 phosphorylation of S6K1. Therefore, during PKC activation, the c-Raf/MEK/extracellular signal-regulated kinase-1/2 (ERK1/2) pathway mediates both the Thr-421/Ser-424 and the Thr-389 phosphorylation in an mTOR-independent and -dependent manner, respectively. Together, our *in vivo* and *in vitro* studies indicate that the PKC/c-Raf/MEK/ERK pathway plays a major role in the S6K1 activation in hypertrophic cardiac growth.

INTRODUCTION

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Hypertrophic cardiac growth is a major compensatory response of the heart to an increased mechanical (hemodynamic) load in the form of either pressure or volume overload. Although this response is initially compensatory, a transition from this state to failure occurs when further growth of the heart is not sufficient to normalize the wall stress and maintain contractile function (1). Therefore, a major research interest in cardiovascular disease is to understand how the increase in hemodynamic load is transmitted intracellularly for mediating hypertrophic growth. Although the mechanical load appears to directly regulate the hypertrophic growth initiation, the signaling mechanism that connects load to such growth is not well understood.

A major cellular event during cardiac hypertrophy is increased protein synthesis (1-5). Enhanced protein synthesis can occur via accelerated protein translation, increased biogenesis of translational components, or both. A significant amount of mRNA of vertebrate cells possesses a unique 5'-terminal oligopyrimidine (5'-TOP)[1] sequence in the 5'-untranslated region (5'-UTR), and these mRNA species generally code for specific ribosomal proteins (6, 7). Their translation is largely controlled via phosphorylation of the 40 S ribosomal S6 protein (S6 protein) at its C terminus (8) by p70/85 S6 kinase (S6K1) (9-12). There are two isoforms of S6K1: the 70-kDa isoform was first isolated from mouse 3T3 cells (13), and the 85-kDa isoform of this kinase was then identified (14). The p85 isoform is expressed from the same transcript as the p70 isoform through an alternative translational initiation start site, which adds a 23-amino acid nuclear localization signal to the N terminus (15, 16). Therefore, the 85-kDa isoform is predominantly in the nucleus, whereas the 70-kDa isoform is present mostly in the cytoplasm. Both the S6K isoforms are collectively called p70/85S6K, p70S6K, or S6K1 and have been shown to phosphorylate the S6 protein and mediate the biogenesis of the translational components, including several of the ribosomal proteins and elongation factors (12). The p85 isoform has been shown to have additional roles in translational control, G_1 to S phase transition, and increased DNA synthesis (17).

Recent studies using S6K1 knockout mice (18) demonstrate no appreciable change in S6 protein phosphorylation, 5'-TOP mRNA translation, or cell growth, although these mice exhibited a small mouse phenotype. These studies (18) and other independent studies (19-21) resulted in the discovery of another S6K (S6K2), which possesses 70% homology with the p70 isoform of S6K1 but is predominantly present in the nucleus due to a C terminus-bound nuclear localization signal.

In the case of S6K1, complex multisite phosphorylations have been shown to occur in a sequential

manner for kinase activation (22-25). At least eight different phosphorylation sites have been identified and can be divided largely into two sets (although the same numbering is used for the position of amino acids in p70 and p85 S6K1 isoforms, 23 residues should be added to convert to the numbering of the p85 isoform). One set of phosphorylation sites, which is important for the kinase activity and sensitivity to rapamycin, has sites in the linker region (Thr-389 and Ser-404 sites) and the catalytic domain (Thr-229 and Ser-371 sites) (22, 26). Thr-389 is located immediately C-terminal to the catalytic domain, and the phosphorylation at this site, which occurs during mitogenic stimulation and is rapamycin-sensitive, is important both for Thr-229 phosphorylation and increased kinase activity. The Thr-229 site is present in the activation loop of the catalytic domain, and phosphorylation at this site is mediated by the 3-phosphoinositide-dependent protein kinase 1 (27). A second set of phosphorylation sites involves four different residues in the pseudosubstrate domain: Ser-411, Ser-418, Thr-421, and Ser-424. Phosphorylation of these residues is important for subsequent Thr-389 phosphorylation as well as kinase function (28). Excluding Ser-411, the phosphorylation of the remaining three residues is mediated independent of the rapamycin-sensitive pathway(s) (26). These phosphorylation sites in the pseudosubstrate domain (also known as autoinhibitory domain) contain the consensus "Ser/Thr-Pro" sequence and, therefore, can be phosphorylated by several members of the proline-directed protein kinases, including the MAPK and cyclin-dependent kinases (29).

At least two major signaling pathways have been described for the phosphorylation and activation of S6K1 (30, 31): a protein kinase C (PKC)-dependent pathway (32, 33), and a PKC-independent pathway that occurs via the activation of phosphatidylinositol 3-OH-kinase (PI3K) (31, 32, 34). A protein kinase A (PKA)-dependent pathway has been recently shown to activate S6K1 (35) and protein kinase B (PKB) (36), a kinase involved in the PI3K-mediated S6K1 activation. Several earlier studies (32, 34, 37) demonstrate that the MAPK family members, such as ERKs, were neither necessary nor sufficient for S6K1 activation. Furthermore, an isoform of PKC (*e.g.* PKCδ) has been shown to associate directly with mTOR (38), indicating the possibility for S6K1 activation without the involvement of c-Raf/MEK/ERK pathway. However, recent reports (39-41) demonstrate the importance of ERK signaling for S6K1 activation under specific conditions.

In 1- to 4-h pressure-overloaded feline myocardium, our recent study (42) showed that the PKC, but not the PI3K-dependent pathway, contributes significantly to S6K activation. In the present study, we observed that this activation was also accompanied by the activation of the c-Raf/MAPK pathway. Therefore, we used cultured adult feline cardiomyocytes or cardiocytes to explore: (i) whether c-Raf, MEK, and MAPKs are key intermediary players for PKC-mediated S6K1 activation, and (ii) whether the PKC- but not the PI3K-mediated S6K1 activation requires the c-Raf/MEK/MAPK pathway. Using dominant negative c-Raf (C4B) adenovirus, we demonstrate for the first time that c-Raf is a critical downstream component for the PKC-mediated, but not the PI3K or the PKA-mediated, phosphorylation of S6K1 and mTOR at their critical sites in adult feline cardiocytes. Furthermore, we demonstrate that the PKC-mediated S6K1 activation and mTOR phosphorylation require the involvement of both MEK1/2 and ERK1/2.

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Chemicals-- Phenylmethylsulfonyl fluoride, 1, 4-dithiothreitol, and E-64 (*trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane) were purchased from Roche Molecular Biochemicals GmbH (Mannheim, Germany). Aprotinin, leupeptin, sodium orthovanadate, okadaic acid, EGTA, Triton X-100, and β-glycerophosphate were obtained from Sigma Chemical Co. (St. Louis, MO); insulin was from Invitrogen (Grand Island, NY); 12-*O*-tetradecanoylphorbol-13-acetate (TPA), forskolin, bisindolylmaleimide-I (BIM), wortmannin, U0126, SB202190 and SB203580 were from Calbiochem (La Jolla, CA).

Antibodies-- The following antibodies were commercially obtained: anti-C-terminal S6K1 (C-18) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA); anti-phospho-Thr-389 S6K1, anti-phospho-Thr-421/Ser-424 S6K1, anti-phospho-PKC (pan), anti-phospho-Thr-202/Tyr-204 ERK1/2, anti-phospho-Thr-180/Tyr-182 p38 MAPK, anti-phospho-Thr-183/Tyr-185 SAPK/JNK (stress activated protein kinase/c-Jun N-terminal kinase), anti-phospho-Ser-235/Ser-236 ribosomal S6 protein, anti-phospho-Ser-2448 mTOR, and anti-phospho-Thr-308 PKB from Cell Signaling Technology Inc. (Beverly, MA); anti-c-Raf from Transduction Laboratory (Lexington, KY).

Animal Model-- Adult cats weighing ~3 kg were used for right ventricular pressure-overload (RVPO) by partial occlusion of the pulmonary artery, as we described previously (43, 44). Briefly, cats underwent partial pulmonary artery occlusion by either external banding for 24 and 48 h or insertion of a balloon catheter for 1 and 4 h. Systemic arterial pressure remained the same whereas the pulmonary arterial pressure was at least doubled. The left ventricle (LV) from each cat served as the same animal internal control for pressure-overloaded right ventricle (RV). Additional control samples (LV and RV) were obtained from sham-operated cats by thoracotomy and pericardiotomy without any arterial occlusions. The care of the animals and all experiments were conducted in accordance with the institutional guidelines of Medical University of South Carolina.

Adult Cardiocyte Culture Model-- Adult feline cardiocytes were isolated from normal cats and cultured on laminin coated four-well culture trays as described previously (45). Isolated cardiocytes were suspended in a 1.8 mM Ca^{2+} containing mitogen-free M-199 medium at pH 7.4. Cells were plated at a density of 1.5×10^5 cells/well and cultured at 37 °C in humidified air with 5% CO_2.

Recombinant Adenoviruses for Dominant Negative c-Raf Expression-- c-Raf dominant negative mutant plasmid (RSV-Raf-C4B) was kindly provided by Dr. M. Abdellatif at the Baylor College Medicine. The plasmid was derived by fusing the N-terminal regulatory domain of c-Raf to the C-terminal antigenic region of B-Raf (46). The dominant negative effect is attributed to a cysteine finger domain in the N-terminal regulatory domain (C4), which interacts with upstream factors resulting in the loss of endogenous c-Raf activation.

RSV-Raf-C4B was cloned into adenovirus shuttle plasmid pAd.CMV-Link.1 (47). Each cDNA insert was subcloned into the multiple cloning site of the shuttle plasmid by standard cloning procedures. The shuttle plasmid contained linker arms flanking either side of the cDNA insert that consists of

adenovirus-5 sequences from 0 to 1 map unit and 9 to 16 map units, respectively. The plasmid was modified previously to produce a high level of expression by placing the constitutively active CMV promoter on the 5'-end of the multiple cloning sites and the SV40 polyadenylation signal on the 3'-end. As a test to demonstrate constitutive expression of the cDNA inserts, the shuttle plasmid was transiently transfected into human 293 kidney cells and protein expression monitored by Western blotting. The purified shuttle plasmid was digested with the restriction enzymes *Nhe*I and *Nar*I to obtain the "rescue" fragment. The fragment was then purified on agarose gel, and 2 μg of purified rescue fragment was used for homologous recombination.

The adenoviral plasmid pTG3602 from the SCS110 bacterial strain was grown, and the purified plasmid was linearized by cutting at the *Cla*I site present in the E1A region. Linearized pTG3602 (10 ng) was then mixed with the rescue fragment, and the DNA mixture was transformed into the BJ5183 bacterial strain and incubated overnight. Colonies were screened by digesting the DNA with *Xho*I and performing a Southern blot to confirm the presence of the cDNA insert. The DNA with the proper orientation and insert was transformed into the $DH5_{\alpha}$ bacterial strain for recombination. The recombinant construct, purified using Qiagen Maxi-preps, was digested overnight with *Pac*I and transfected into 293 cells using LipofectAMINE. The 293 cell line has the E1 region integrated into its genome; the defective E1 region of the adenoviral genome was complemented by the cellular genome.

Adenoviral plaques were allowed to develop, and the plaque was purified a second time by infecting 293 cells and overlaying with agar. After large-scale preparation, adenoviruses were purified by CsCl gradient centrifugation, dialyzed, and titered by plaque assay (48, 49). Adenovirus for mitogen-activated protein kinase phosphatase-3 (MKP-3), generated using similar protocol, was obtained from Dr. Donald R. Menick's laboratory.

Stimulation of S6K1 in Cultured Cardiocytes-- Freshly isolated adult feline cardiocytes were cultured overnight and stimulated with 200 nM TPA, 100 nM insulin, or 1 μM forskolin in the presence or absence of various pharmacological agents for indicated periods of time. Stock solutions for TPA and forskolin were prepared in Me_2SO, and Me_2SO-treated cardiocytes were used as controls for these experiments. For treatment with pharmacological inhibitors, cardiocytes were preincubated for 30 min with the inhibitors and then the cells were stimulated with TPA, insulin, or forskolin. For the adenoviral expression, freshly isolated cardiocytes were plated on laminin-coated trays and incubated for 4 h prior to infection. Cells were then incubated overnight in serum-free M-199 media containing the adenovirus at m.o.i. (multiplicity of infection) levels of 2 and 10 for MKP-3 and 250 for C4B. Cells infected with an equal m.o.i. of β-galactosidase adenovirus served as control. The media was replaced with serum-free M199 media, and cells were incubated for an additional 24 h before agonist stimulation.

Western Blotting-- Triton X-100 soluble and insoluble samples were prepared as we previously described (50) with a few minor modifications. Briefly, following the stimulation, cardiocytes were extracted with lysis buffer (30 mM Tris-HCl, pH 7.4, 2% Triton X-100, 10 mM β-glycerolphosphate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 μM E-64, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 0.02 μM okadaic acid, 0.5 mM EGTA). The cell lysate was centrifuged at 160,000 × *g* for 30 min, and the protein concentration in the supernatant was measured using BCA

(Pierce) reagent. The supernatant was then boiled with SDS-sample buffer and used as detergent-soluble fraction. The pellet obtained after centrifugation was resuspended in SDS-sample buffer, boiled for 5 min, and centrifuged for 5 min at room temperature to obtain detergent-insoluble fraction. The protein concentration in each sample was determined using BCA reagent (Pierce) and adjusted for comparison.

20 μl of detergent-lysed subfractions (or insoluble fraction in the case of S6 protein detection) was resolved by SDS-PAGE, and the proteins were transferred electrophoretically to Immobilon-P membranes (Millipore Corp., Bedford, MA). The membranes were blocked for 1 h using 10% milk in TBST buffer (10 mM Tris, 0.1 M NaCl, 0.1% Tween 20, pH 7.4). Blots were incubated with the primary antibodies in TBST buffer overnight at 4 °C with gentle agitation. Following the incubation with the primary antibodies, blots were washed three times with TBST buffer each for 5 min and incubated with appropriate horseradish peroxidase-labeled secondary antibodies (Vector Laboratories, Burlingame, CA) in TBST buffer for 1 h at room temperature. After washing the blots, the proteins were detected by enhanced chemiluminescence (Renaissance, PerkinElmer Life Sciences, MA).

RESULTS

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S6K1 Activation in Pressure Overloaded Myocardium-- The purpose of this study was to demonstrate that the PKC-mediated S6K1 activation in adult cardiocytes requires the activation of the c-Raf/MEK/MAPK pathway. To test this possibility, we analyzed whether the S6K1 phosphorylation/activation in pressure-overloaded feline myocardium is accompanied by the activation of MAPK family members (ERK1/2, p38 MAPK, and JNKs), c-Raf, and PKC. The activation of all these kinases can be analyzed by taking three different approaches: (i) Western blot analysis using regular antibodies to observe changes in the mobility on SDS-PAGE, which is indicative of distinct phosphorylation of these kinases upon activation, (ii) Western blot analysis using phospho-specific antibodies to determine the phosphorylation of critical residues necessary for kinase activation, and (iii) immune complex kinase assays. In the present study, we undertook the first two approaches.

Right ventricular pressure overload was induced for time periods of 1-48 h by pulmonary artery occlusion. Sham-operated control cats underwent similar surgical interventions without occluding the pulmonary artery. Compared with the sham control ventricles (LV or RV) or unloaded same animal LV controls, 1-h pressure-overloaded RVs exhibited a dramatic change in the mobility of both the S6K isoforms (p70 and p85) during SDS-PAGE separation (Fig. 1*A*). This retarded mobility (band shifting) was also seen in 4- and 24-h pressure overloaded RV, and returned partially to the control levels in 48 h RV. We used phospho-specific antibodies that detect either the Thr-389-phosphorylated or the Thr-421/Ser-424-phosphorylated (simultaneously) S6K1 isoforms. Thr-389 and Thr-421/Ser-424 phosphorylation of the p70 S6K isoform was either almost absent or present at very low levels in unloaded control LVs and RVs. However, 1-h pressure-overloaded RVs exhibited a robust increase in phosphorylation of all three sites. Such increased phosphorylation was also observed in 4-, 24-, and 48-h pressure-overloaded RVs. In the case of the p85 isoform, Thr-389 phosphorylation was significant in 4- and 24-h pressure-overloaded myocardium, whereas for the Thr-421/Ser-424 sites, significant

phosphorylation was observed as early as 1 h and persisted up to at least 48 h of pressure overloading. The pattern of S6K1 activation matches precisely with our earlier studies performed under similar conditions (42). Overall, these data demonstrate once again that S6K1 is activated to a substantial level as early as 1 h of pressure overloading of the myocardium, and the activation is sustained for at least 24 h. Interestingly, the S6K1 activation is also accompanied by an increased mTOR phosphorylation at the Ser-2448 site. Such enhanced phosphorylation, which is indicative of mTOR activation (51), was observed as early as 1 h of pressure overload and matches the S6K1 activation time course.

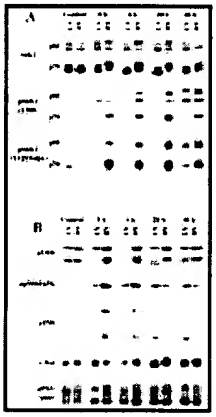

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Fig. 1. Activation of S6K1 and PKC/c-Raf/MAPK pathway in pressure-overloaded myocardium. Left and right ventricles (LVs and RVs) obtained from sham-operated control feline hearts and from 1-, 4-, 24-, and 48-h right ventricular pressure-overloaded (RVPO) feline hearts were homogenized in Triton X-100 buffer. The Triton-soluble fraction was then used for Western blot analysis using non-selective and phosphorylation state-specific antibodies as described under "Materials and Methods." *A*, Western blot showing electrophoretic mobility change, detected with non-selective anti-S6K1 antibody and phosphorylation status at specific sites (Thr-389 and Thr-421/Ser-424) of S6K1 isoforms, detected with phosphorylation state-specific antibodies. *B*, Western blot showing changes in electrophoretic mobility of c-Raf (measured using non-selective anti-c-Raf antibody) and phosphorylation status (measured using phosphorylation state-specific antibodies) of ERK1/2, p38 MAPK, JNK, and PKC. The results were confirmed in two other independent sets of experimental cats.

Next, we analyzed whether a signaling pathway that includes the activation of PKC, c-Raf, and the MAPK family members accompanies the S6K1 activation process. The activation of MAPK family members and PKC was determined by Western blot analysis using phospho-specific antibodies whereas c-Raf activation was analyzed by its retarded electrophoretic mobility during SDS-PAGE separation combined with Western blot detection. Analysis using phospho-specific antibodies for extracellular signal-regulated kinase (ERK isoforms, 42 and 44 kDa), p38 MAPK, and c-Jun N-terminal kinase (JNK isoforms, 42 and 60 kDa) showed a clear activation of all of these family members in 1- to 4-h pressure-overloaded right ventricular samples (Fig. 1*B*). Whereas both of the ERK isoforms remained active up to 48 h, p38 MAPK and JNK showed a decline in phosphorylation in 24- and 48-h pressure-overloaded myocardium following their initial activation. Furthermore, pressure overloading for 1 h or more resulted in a retarded electrophoretic mobility of c-Raf (disappearance of the lower band) during SDS-PAGE separation, which is indicative of phosphorylation and activation as reported previously (52). To demonstrate PKC activation, we used a commercially available phospho-specific pan PKC antibody. Although the antibody detected protein bands even in the unloaded controls (*lower most band* is present both in LV or RV of control cat sample), pressure-overloaded RV samples, relative to the unloaded (same animal) LV control, exhibited one or more newly phosphorylated PKC isoforms (*upper bands*) indicative of their activation. The time course of activation of the PKC/c-Raf/MAPK pathway and S6K1 is very similar.

Agonist-stimulated S6K1 Activation in Adult Cardiocytes-- Cardiocytes cultured for 24 h were stimulated with 200 nM TPA, 100 nM insulin, or 1 μM forskolin to activate PKC, PI3K, or PKA, respectively. Treatment with all three agents activated both p70 and p85 isoforms (S6K1) within 30 min as evidenced by the retarded electrophoretic mobility during SDS-PAGE separation (Fig. 2*A*). Furthermore, studies performed with phospho-specific antibodies demonstrated that stimulation of cardiocytes with all three agents resulted in the Thr-389 phosphorylation of both the S6K1 isoforms (p70 and p85). This phosphorylation was observed as early as 8 min and peaked within 30 min of treatment. However, Thr-421/Ser-424 phosphorylation was significantly increased over control only in TPA-treated cells. Furthermore, TPA-stimulated phosphorylation at Thr-421/Ser-424 sites (8 min) was found to occur prior to the phosphorylation at the Thr-389 residue (15 min) and remained higher up to 60 min. This phosphorylation pattern of the Thr-389 and Thr-421/Ser-424 residues in S6K1 isoforms following the TPA treatment appeared similar to the changes observed in pressure-overloaded myocardium (Fig. 1*A*).

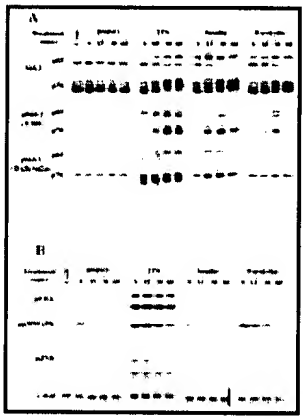

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Fig. 2. Agonist-stimulated activation of S6K1 and c-Raf/MAPK pathway in isolated adult feline cardiocytes. Adult feline cardiocytes were cultured on laminin-coated plates overnight and stimulated with 200 nM TPA, 100 nM insulin, or 1 μM forskolin for various time periods. Cardiocytes left either in media alone (*Cont*) or in Me_2SO (*DMSO*) served as controls for insulin and for TPA and forskolin, respectively. Cells were processed as described under "Materials and Methods," and the samples were used for Western blot analysis using non-selective and phosphorylation state-specific antibodies. *A*, Western blot showing electrophoretic mobility change and phosphorylation state at specific sites of S6K1 isoforms. *B*, Western blots showing changes in electrophoretic mobility of c-Raf and phosphorylation status of ERK1/2, p38 MAPK, and JNK. The results were confirmed in two other independent experiments.

Next, we analyzed whether the agonist-stimulated S6K1 activation was accompanied by the activation of one or more c-Raf/MEK/MAPK pathways. Compared with the untreated or Me_2SO-treated controls, TPA-treated cardiocytes exhibited a substantial phosphorylation/activation of ERK1/2 (both the p42 and p44 isoforms) and p38 MAPK (Fig. 2*B*). However, TPA treatment did not result in a significant activation of JNK family kinases (both p42 and p60 isoforms). In the Western blot performed for JNK, a low level protein band in the TPA panel actually corresponds to ERK1/2 and not JNK. Apparently, this might be due to cross-reactivity of the phospho-specific JNK antibody. In the case of the other two stimulants, there were no significant changes observed for all the MAPK family members following the insulin treatment, although forskolin treatment resulted in p38 MAPK activation. Furthermore, c-Raf showed retarded electrophoretic mobility only in TPA-treated cardiocytes (Fig. 2*B*, *TPA panel*), and this observation was similar to that seen in pressure-overloaded myocardium. However, all three stimulants, similar to their effect on S6K1, caused phosphorylation of mTOR at the Ser-2448 site, indicative of its activation (51). Overall, these results demonstrate that only in the TPA-stimulated cardiocytes, S6K1

activation is accompanied by the activation of c-Raf/ERK signaling pathway and that this activation pattern matches the observation in *in vivo* pressure-overloaded myocardium.

Specificity of TPA and Insulin-stimulated S6K1 Activation-- We analyzed whether TPA, insulin, and forskolin activate S6K1 via stimulating PKC, PI3K, and PKA, respectively. For this, we used specific pharmacological inhibitors, bisindolylmaleimide-I (BIM) and wortmannin to block PKC and PI3K, respectively. Pretreatment of cardiocytes with 5 μM BIM resulted in the loss of the TPA-stimulated but not the insulin- or forskolin-stimulated S6K1 phosphorylation/activation (Fig. 3). On the other hand, 100 nM wortmannin had no effect on TPA- and forskolin-stimulated S6K1 activation but significantly blocked the insulin-stimulated S6K1 activation, as evidenced by a faster electrophoretic mobility and reduced Thr-389 phosphorylation. These data indicate that S6K1 activation during TPA, insulin, and forskolin stimulation is mediated via PKC, PI3K, and PKA pathways, respectively.

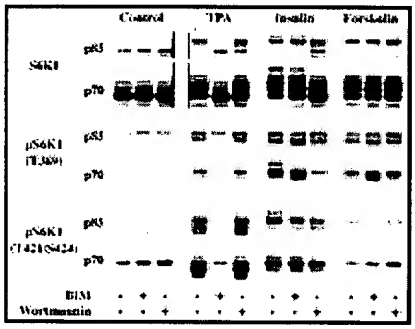

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Fig. 3. Demonstration of S6K1 activation via PKC, PI3K, or PKA pathway during various agonist stimulation. Cultured adult cardiocytes were preincubated for 30 min in the absence or presence of 5 μM bisindolylmaleimide-I (*BIM*) or 100 nM wortmannin and then stimulated for 30 min with 200 nM TPA, 100 nM insulin, 1 μM forskolin, or control. Cells were processed as described under "Materials and Methods," and the samples were used for Western blot analysis using non-selective and phosphorylation state-specific S6K1 antibodies. The results were confirmed in one other independent experiment.

Effect of Dominant Negative c-Raf on S6K1 Activation-- A major interest of this study is to demonstrate that, during PKC-mediated S6K1 activation, c-Raf plays a critical intermediate role. Both in pressure-overloaded myocardium and in TPA-stimulated adult cardiocytes, S6K1 activation was accompanied by a retarded mobility of c-Raf during SDS-PAGE separation, indicative of its kinase activation.

We constructed a recombinant adenovirus, because the conventional transfection is not possible in adult cardiocytes, to facilitate the expression of dominant negative c-Raf mutant (C4B). Adenovirus harboring the β-galactosidase gene was used as a control virus. Cardiocytes were infected with 250 m.o.i. of adenovirus, a concentration determined to be sufficient to block the c-Raf-mediated effect. In C4B adenovirus-infected cardiocytes, but not in β-galactosidase adenovirus-infected cardiocytes, C4B protein was expressed in substantial amounts (Fig. 4*A*), at least 20-fold higher than the endogenous c-Raf level (the same antibody was used to detect the 32-kDa C4B and 65-kDa c-Raf). TPA treatment of control or β-galactosidase-expressing cells resulted in the retarded electrophoretic mobility of c-Raf, as observed earlier (Fig. 2*B*). However, such effect was completely lost in the case of C4B-expressing cells and showed faster migration, indicating the loss of c-Raf activation (Fig. 4*A*). c-Raf activation was low in insulin- and forskolin-treated cardiocytes, and the expression of dominant negative c-Raf blocked even the low level activity of c-Raf as indicated by the faster mobility of this kinase during SDS-PAGE

separation.

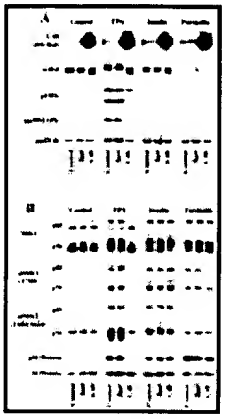

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Fig. 4. Effect of dominant negative c-Raf (C4B) on agonist-stimulated S6K1 activation in adult cardiocytes. Freshly isolated adult cardiocytes cultured on laminin-coated plates were infected overnight with 250 m.o.i. (multiplicity of infection) of an adenovirus harboring either β-galactosidase or C4B as described under "Materials and Methods." 36 h post infection, the cardiocytes were either left untreated (control) or stimulated with 200 nM TPA, 100 nM insulin, or 1 μM forskolin. Cells were processed as described under "Materials and Methods," and the samples were used for Western blot analysis using non-selective and phosphorylation state-specific antibodies. *A*, Western blot showing expression of C4B, changes in c-Raf electrophoretic mobility and phosphorylation status of ERK1/2, p38 MAPK, and mTOR. *B*, Western blot showing electrophoretic mobility change and phosphorylation status at specific sites of S6K1 isoforms and S6 protein. The results were confirmed in three other independent experiments.

Next, we analyzed whether the expression of dominant negative c-Raf (C4B) results in the loss of MAPK activation. Compared with the control and insulin- and forskolin-treated cells, 30-min TPA treatment caused both ERK1/2 and p38 MAPK activation (Fig. 4*A*), as was observed in the previous experiment (Fig. 2*B*). Results shown in Fig. 4A demonstrate clearly that C4B expression blocks the activation of TPA-stimulated ERK1/2 and p38 MAPK activation. Therefore, expression of C4B at ~20-fold higher the concentration of endogenous c-Raf can result in the loss of TPA-mediated activation of c-Raf, ERK1/2, and p38 MAPK. Although forskolin-stimulated p38 MAPK activation was observed in the previous experiment (Fig. 2*B*), its activation was low in the present experiment. Furthermore, forskolin stimulation, but not TPA or insulin-stimulation, lowered the c-Raf level in these long term cultured (a total of 3 days) cardiocytes, used for adenoviral infection.

We also analyzed the phosphorylation status of mTOR using a Ser-2448 phosphorylation state-specific antibody. All three stimulants phosphorylated mTOR at the Ser-2448 site (Fig. 4*A*). C4B expression specifically blocks the TPA-stimulated but not the insulin- or forskolin-stimulated mTOR phosphorylation. Therefore, c-Raf activity is required only for the TPA-stimulated mTOR activation.

Because the expression of dominant negative c-Raf resulted in the loss of the c-Raf/MAPK pathway, we next analyzed the importance of this pathway for S6K1 activation. Expression of dominant negative c-Raf (C4B) almost completely blocked the TPA-stimulated changes in the electrophoretic mobility of S6K1 isoforms (both p70 and p85), as well as blocked the phosphorylation at Thr-389 and Thr-421/Ser-424 sites (Fig. 4*B*). Importantly, this effect is very specific to TPA-stimulated S6K1 activation, because the insulin-stimulated S6K1 activation is unaffected under these conditions. Similarly, in forskolin-stimulated cardiocytes, the retarded electrophoretic mobility was not affected by the expression of C4B, although a low level reduction was observed in Thr-389 phosphorylation.

To demonstrate further that C4B expression blocks S6K1 activation in TPA-stimulated cells, we

analyzed the phosphorylation state of 40 S ribosomal S6 protein, a specific target of S6K1 isoforms (9-12). Stimulation of cardiocytes with all the stimulants resulted in a substantial increase in the level of phosphorylated S6 protein (Fig. 4*B*). However, C4B, but not the control (β-galactosidase) adenovirus-infected cardiocytes, showed a complete loss of TPA-stimulated S6 protein phosphorylation. Similar to the effect on S6K1, S6 protein phosphorylation was unaffected during insulin or forskolin stimulation. Therefore, all these studies clearly demonstrate that the dominant negative c-Raf specifically blocks the TPA-stimulated (PKC-mediated) S6K1 activation and S6 protein phosphorylation and that these events are unaffected during insulin or forskolin stimulation.

Effect of MEK1/2 Inhibitor (U0126) on S6K1 Activation-- A major downstream target of c-Raf is mitogen-activated protein kinase kinase/ERK kinase (MEK1/2), which is an immediate upstream activator of ERK1/2. We employed U0126, a specific inhibitor of MEK1/2, and analyzed whether pretreatment with this drug blocks TPA-stimulated S6K1 activation. The effect of U0126 on TPA-stimulated S6K1 activation mirrors the C4B (dominant negative c-Raf) effect. The TPA-stimulated activation of both the S6K1 isoforms (p70 and p85), as measured in terms of either band-shifting or phosphorylation at Thr-389 and Thr-421/Ser-424 sites, was significantly blocked when cardiocytes were preincubated with 10 μM U0126 (Fig. 5). However, unlike the C4B that blocked only the TPA effect, the U0126 treatment showed a low level blocking effect on the insulin-stimulated S6K1 activation (as evidenced by the partial reversal of both the electrophoretic mobility and the loss of phosphorylation at Thr-389 and Thr-421/Ser-424 sites). However, the changes associated with S6K1 during forskolin treatment were unaffected by the drug treatment.

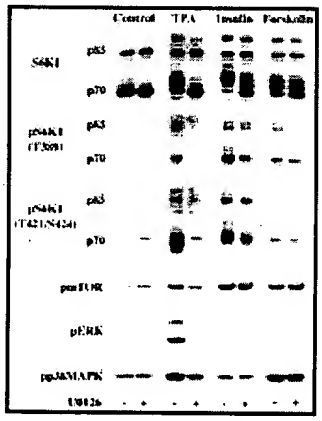

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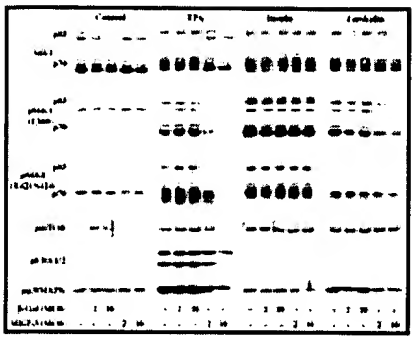
Fig. 5. Effect of MEK1/2 inhibitor (U0126) on agonist-stimulated S6K1 activation in adult cardiocytes. Cultured adult cardiocytes were preincubated for 30 min in the absence or presence of 10 μM U0126 and then stimulated for 30 min with 200 nM TPA, 100 nM insulin, or 1 μM forskolin. Unstimulated cardiocytes served as controls. Cells were processed as described under "Materials and Methods," and the samples were used for Western blot analysis using non-selective and phosphorylation state-specific antibodies. Western blot shows electrophoretic mobility change and phosphorylation status at specific sites of S6K1 isoforms, mTOR, ERK1/2, and p38 MAPK. The results were confirmed in two other independent experiments.

Treatment of cardiocytes with U0126 blocked only the TPA-stimulated but not the insulin- or forskolin-stimulated mTOR phosphorylation. This suggests that the c-Raf/MEK pathway is important for the Ser-2448 phosphorylation of mTOR. We also tested whether U0126 treatment results specifically in the loss of ERK1/2 activation but not the other members of the MAPK family such as the p38 MAPK. Pretreatment of cardiocytes with U0126 strongly blocked the TPA-stimulated ERK1/2 activation (Fig.

5). However, its blocking effect on the TPA-stimulated p38 MAPK activation was weak. As observed in the previous experiment (Fig. 2*B*), only forskolin treatment but not insulin treatment resulted in p38 MAPK activation. However, such activation was not significantly altered by the pretreatment with U0126. Overall, these data demonstrate that U0126 treatment, which is known to block the MEK1/2 activation, results in the loss of TPA-stimulated ERK1/2 activation and mTOR phosphorylation, and these effects are very similar to C4B effect in TPA-stimulated cardiocytes. The loss of these kinase activities is observed with the loss of S6K1 phosphorylation and activation, demonstrating that the c-Raf/MEK pathway is critical for the TPA-mediated S6K1 activation.

Effect of ERK1/2-specific Phosphatase (MKP-3) Expression on S6K1 Activation-- Next, we analyzed the importance of ERK1/2 as a downstream component of c-Raf/MEK signaling in mediating S6K1 activation. Because no specific pharmacological inhibitors or dominant negative constructs have been developed for blocking ERK1/2, we used MKP-3, a dual specific ERK1/2 phosphatase, to dephosphorylate and inactivate ERK1/2 (53) in an adenoviral construct similar to that for C4B. The infection of cardiocytes was performed as described under "Materials and Methods." We determined the concentration of adenovirus sufficient to block primarily the TPA-stimulated ERK and S6K1 activation. Infection of cardiocytes with 2 m.o.i. of MKP-3 adenovirus but not β-galactosidase adenovirus lowered the TPA-stimulated ERK1/2 activation, and this effect was observed more significantly at 10 m.o.i. of MKP-3 adenovirus (Fig. 6). This suggests that MKP-3 inactivates ERK1/2, as reported previously (54). Next we analyzed the effect of MKP-3 expression on the agonist-stimulated S6K1 activation. The TPA-stimulated retarded electrophoretic mobility of both S6K1 isoforms was substantially blocked in cardiocytes that were infected with the low concentration of MKP-3 adenovirus (2 m.o.i.) but not in β-galactosidase virus-infected cardiocytes. At the higher concentration of MKP-3 adenovirus (10 m.o.i.) but not β-galactosidase adenovirus, the change in TPA-stimulated electrophoretic mobility is almost completely lost. However, such a change in electrophoretic mobility in insulin- and forskolin-stimulated cardiocytes was not significantly affected by MKP-3 expression. These data suggest that ERK1/2 inactivation has a profound effect on the TPA-stimulated S6K1 activation. Analysis of the phosphorylation status of S6K1 demonstrates that both the Thr-421/Ser-424 and Thr-389 phosphorylation was significantly lost in TPA-stimulated cells. However, MKP-3 expression even with higher concentration of adenovirus showed no effect on the insulin-stimulated S6K1 phosphorylation, although a low level blocking effect was observed on the forskolin-stimulated S6K1 phosphorylation. Furthermore, the effect of MKP-3 on the mTOR phosphorylation was similar to the loss of ERK1/2 and S6K1 phosphorylation. That is, Ser-2448 phosphorylation of mTOR was specifically reduced in the case of TPA- but not insulin- or forskolin-stimulated cardiocytes. These studies demonstrate that MKP-3 expression, which is used to block ERK1/2, results in a significant loss on the TPA-stimulated S6K1 and mTOR phosphorylation, and these effects are similar to the effect observed with C4B expression. Although the expression of MKP-3, an ERK1/2-specific phosphatase (53), results in the loss of ERK1/2 activation, this phosphatase also lowers p38 MAPK phosphorylation, especially at higher levels of expression.

Fig. 6. Effect of MAPK phosphatase (MKP-3) overexpression on agonist-stimulated S6K1 activation in adult cardiocytes. Freshly isolated adult cardiocytes cultured



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on laminin-coated plates were either left in serum-free media or infected overnight with either MKP-3 or β-galactosidase adenovirus at indicated virus concentration (m.o.i.). 36 h post infection, both the cardiocyte cultures were stimulated with 200 nM TPA, 100 nM insulin, or 1 μM forskolin. The samples were processed as described under "Materials and Methods" and used for Western blot analysis using non-selective and phosphorylation state-specific antibodies. Western blot shows electrophoretic mobility change and phosphorylation status at specific sites of S6K1 isoforms, mTOR, ERK1/2, and p38 MAPK. The results were confirmed in two other independent experiments.

Effect of p38 MAPK Inhibition on S6K1 Activation-- Because our data demonstrate that c-Raf/MEK/MAPK controls PKC-mediated S6K1 activation, we analyzed the importance of MAPK family members. Both U0126 that blocked the MEK/ERK activation and MKP-3 that blocked ERK1/2 activation, showed a partial effect on the p38 MAPK activation. Therefore, it is possible that either ERK1/2 and/or p38 MAPK contribute to the S6K1 activation. p38 MAPK can be blocked with specific SB compounds, such as SB203580 and SB202190. Pretreatment of cardiocytes with these drugs exhibited the following changes (Fig. 7): (i) no significant changes in the TPA-stimulated S6K1 activation and Thr-421/Ser-424 phosphorylation except a low level drop in the Thr-389 phosphorylation, (ii) insulin-stimulated S6K1 activation (retarded electrophoretic mobility) and Thr-389, Thr-421/Ser-424 phosphorylation were partially blocked, and (iii) forskolin-stimulated S6K1 activation and phosphorylation were unaffected. Therefore, in the case of TPA-stimulated S6K1 activation, the phosphorylation of Thr-421/Ser-424 sites for which p38 MAPK could serve as a potential upstream kinase, is unaffected by the action of SB compounds. Furthermore, although TPA- and insulin-stimulated Thr-389 phosphorylation was partially blocked by SB compounds, mTOR phosphorylation was not affected significantly, especially in the case of TPA- and forskolin-stimulated cardiocytes, indicating mTOR activation alone is not sufficient for the Thr-389 phosphorylation. Overall, these studies performed with SB compounds demonstrate that p38 MAPK does not contribute significantly to S6K1 activation and Thr-421/Ser-424 phosphorylation.

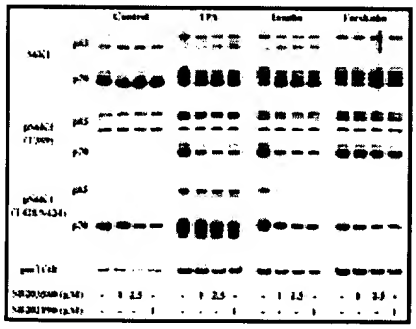

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Fig. 7. Effect of p38 MAPK inhibitors (SB203580 and SB202190) on agonist-stimulated S6K1 activation in adult cardiocytes. Cultured adult cardiocytes were preincubated for 30 min in the absence or presence of SB203580 compound (1 and 2.5 μM) and SB202190 compound (1 μM) and then stimulated for 30 min with 200 nM TPA, 100 nM insulin, or 1 μM forskolin. Unstimulated cardiocytes served as controls. Samples were used for Western blot analysis using non-selective S6K1 antibody and phosphorylation state-specific S6K1 and mTOR antibodies. Western blot shows electrophoretic mobility change and phosphorylation status at specific sites of S6K1 isoforms and mTOR. The

results were confirmed in two other independent experiments.

Effect of Rapamycin on S6K1 Activation-- mTOR is a Ser/Thr kinase and a critical component for S6K1 activation. The functional role of this kinase can be blocked with nanomolar concentrations of rapamycin. Therefore, we analyzed the effect of rapamycin on the phosphorylation pattern of S6K1 during all three types of stimulants. The activation of both S6K1 isoforms by all three stimulants was blocked when cardiocytes were pretreated with rapamycin (Fig. 8). Furthermore, rapamycin treatment blocked Thr-389 phosphorylation, indicating that the phosphorylation at this site requires mTOR activity. Interestingly, rapamycin pretreatment did not affect the TPA-stimulated Thr-421/Ser-424 phosphorylation, although there were changes in the migration of such phosphorylated S6K1 species during SDS-PAGE separation. The changes in the position of Thr-421/Ser-424 phosphorylated protein bands upon rapamycin pretreatment are likely due to faster electrophoretic mobility of S6K1 following the loss of phosphorylation at other potential sites. In the case of insulin-treated cardiocytes, this phosphorylation, which was relatively lower when compared with the TPA-treated cardiocytes, was brought to the basal level by the rapamycin pretreatment. Taken together, these data demonstrate that (i) the Thr-389 phosphorylation induced by all three agents can be blocked with rapamycin and (ii) the phosphorylation at Thr-421/Ser-424 sites of S6K1 in TPA-treated cardiocytes proceeds independent of mTOR.

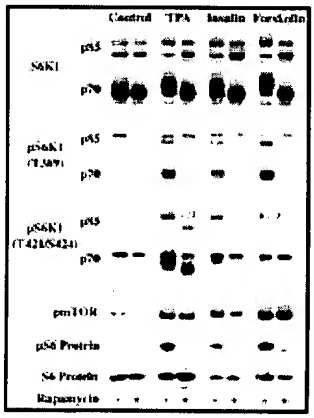

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Fig. 8. Effect of rapamycin on agonist-stimulated S6K1 activation in adult cardiocytes. Cultured adult cardiocytes were preincubated for 30 min in the absence or presence of 2 nM rapamycin and then stimulated for 30 min with 200 nM TPA, 100 nM insulin, or 1 μM forskolin. Unstimulated cardiocytes served as controls. Samples were used for Western blot analysis using non-selective and phosphorylation state-specific antibodies for S6K1, mTOR, and S6 protein. Western blot shows electrophoretic mobility change and phosphorylation status at specific sites of S6K1 isoforms, mTOR, and S6 protein. The level of S6 protein is also determined by analyzing with a non-selective anti-S6 protein antibody. The results were confirmed in two other independent experiments.

Rapamycin treatment did not block the phosphorylation of mTOR at the Ser-2448 site significantly, indicating that the drug affects only the activity of mTOR but not the upstream kinase that is responsible for the mTOR phosphorylation at the Ser-2448 site.

DISCUSSION

Ribosomal biogenesis is an important cellular event for mediating

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hypertrophic cardiac growth (1-5), and the activation of S6K1 is critical for the augmented ribosomal biosynthesis (9-12, 55). S6K1 phosphorylates ribosomal protein S6, which is a key component in the translation of subset mRNA transcripts that possesses a tract of pyrimidines (5'-TOP mRNAs) in the 5'-untranslated region (5'-UTR) (6, 7, 23). 5'-TOP mRNAs generally encode ribosomal proteins and elongation factors, and therefore, the overall translational capacity of cells increases substantially following S6K1 activation. In 48-h pressure-overloaded myocardium, accelerated protein synthesis can be observed with an enhanced new steady-state level (43). Therefore, initial S6K1 activation and ribosomal biogenesis are major myocardial cellular events for sustained hypertrophic growth. The importance of S6K1 activation during pressure overload hypertrophy has been shown recently using angiotensin type II-receptor knockout mice (56). These mice were found to have reduced levels of p70S6K and an absence of pressure overload-induced hypertrophic cardiac growth. The link between S6K1 activation and cell growth has been well documented in other cell types using rapamycin (10, 11, 25, 57) and through microinjection of neutralizing antibodies into cells (15, 17) both of which selectively suppressed S6K1 activation and impeded cell growth. S6K1 activation has been demonstrated in hypertrophying myocardium (42, 58) and in cardiocytes stimulated with hypertrophic agents (39, 59-61). However, the major signaling pathway for S6K1 activation and its importance in the context of hypertrophic growth has not been studied in detail.

The S6K1 activation process, which relies on the sequential interplay between multiple phosphorylation sites and signal transduction pathways (62-64), is complex and not completely understood. Several independent signaling pathways have been identified for S6K1 activation depending upon cell types and the nature of the stimulants (30-36), and these pathways are activated subsequent to at least three independent agonists' stimulation: (i) TPA that activates a PKC-dependent pathway, (ii) insulin that activates a PI3K-dependent pathway, and (iii) forskolin that activates a protein kinase A (PKA)-dependent pathway. Activation of all these pathways has been demonstrated in pressure-overloaded myocardium by several research groups (58, 61, 65-67), although our earlier study (42) demonstrated that the S6K1 activation was accompanied by the activation of PKC but not PI3K pathway in 1- to 4-h pressure overload myocardium. Therefore, we used cultured adult feline cardiocytes to explore the importance of potential downstream players of PKC, namely, c-Raf, MEK, and MAPK family members for S6K1 activation.

Our study demonstrates that stimulation of adult cardiocytes with TPA results in S6K1 activation, which is accompanied by the activation of c-Raf as well as the MAPK family members, ERK1/2 and p38 MAPK, but not JNK. It has been well established that the c-Raf-mediated ERK1/2 activation occurs via the activation of MEK1/2, and our studies with U0126 confirm that this kinase is active in TPA-stimulated cardiocytes. However, stimulation with insulin and forskolin, although they phosphorylate and activate S6K1 and mTOR substantially, did not result in the activation of c-Raf and ERK1/2. Therefore, the contribution of the c-Raf/MEK/ERK pathway to S6K1 activation can be expected mostly during TPA stimulation but not with the other two stimulants.

In the case of S6K1 phosphorylation, although Thr-389 phosphorylation was observed during all three

types of stimulations, the extent of Thr-421/Ser-424 phosphorylation was stronger in TPA-stimulated cardiocytes. Furthermore, in TPA-stimulated cardiocytes, the Thr-421/Ser-424 phosphorylation occurs prior to the Thr-389 phosphorylation, indicating the possibility that the pseudosubstrate phosphorylation occurs first to facilitate the phosphorylation of Thr-389, as suggested previously (68). However, in forskolin-stimulated cardiocytes, the basal level phosphorylation at the Thr-421/Ser-424 sites appears to be sufficient for the Thr-389 phosphorylation. Both PKA and PI3K activation have been demonstrated in pressure-overloaded myocardium (58, 61, 67), indicating the possibility that they contribute to S6K1 activation. However, our findings suggest that PKC-mediated signaling contributes significantly to the S6K1 activation for the following reasons: (i) only in TPA-, but not insulin- or forskolin-treated cardiocytes, the Thr-421/Ser-424 phosphorylation occurs similar to that with pressure overload, (ii) our previous study (42) demonstrates that S6K1 activation in 1- to 4-h pressure-overloaded feline myocardium was not accompanied by PI3K activation, (iii) similar to pressure-overloaded myocardium, TPA-stimulated cardiocytes demonstrate S6K1 activation and phosphorylation, accompanied by the phosphorylation/activation of c-Raf and MAPK (ERK1/2 and p38 MAPK), and (iv) analysis of PKB, a downstream kinase of PI3K, in 1-h pressure-overloaded RV did not show appreciable change in its phosphorylation level when compared with the unloaded control LV (data not shown). Therefore, a major focus of this study was to determine the key intermediary players responsible for the PKC-mediated S6K1 activation in adult feline cardiocytes.

Whereas previous studies have shown that Raf/MAPK signaling is neither necessary nor sufficient for S6K1 activation by mitogens (34, 69, 70), our data support recent studies demonstrating that the expression of either active c-Raf or MEK is sufficient for S6K1 activation in an ERK-dependent manner (29, 37, 71). Furthermore, MEK has been shown to activate a closely related S6K1 member, S6K2, in adult rat cardiocytes (39), although the importance of this isoform has not been studied in detail in pressure-overloaded myocardium. MEK1/2, but not ERK1/2, has been shown to be important for S6K1 activation in response to insulin or phorbol esters in adipocytes (72, 73). Therefore, *in vivo* activation of either c-Raf or MEK alone appears to be sufficient for S6K1 activation, although the importance of ERK1/2 in this process is not clear.

To explore the possibility that the c-Raf/MEK/MAPK pathway contributes to the PKC-mediated S6K1 activation, we used the dominant negative approach to block c-Raf, the pharmacological agents to block the MEK, and the overexpression of a specific phosphatase to block ERK. Expression of C4B, resulting in dominant negative c-Raf concentrations ~20-fold higher than the endogenous levels, blocked the TPA-stimulated activation of c-Raf, ERK1/2, and p38 MAPK. Whereas C4B expression abolished the TPA-stimulated phosphorylation of S6K1 and S6 protein, it does not exhibit any such effect in the insulin- and forskolin-stimulated S6K1 activation and S6 protein phosphorylation. This observation suggests that: (i) the insulin- and forskolin-stimulated S6K1 activation, which is known to occur via PI3K and PKA activation, respectively, is independent of the PKC-stimulated pathway that requires c-Raf, and (ii) adenoviral expression of C4B specifically blocks the c-Raf-mediated effect and does not have nonspecific effects on other signaling pathways. Taken together, these studies indicate that c-Raf is a specific downstream player of PKC during TPA-stimulated S6K1 activation.

Similar to the C4B experiment, U0126 treatment blocks the TPA-induced phosphorylation of S6K1 and

mTOR. This clearly suggests that MEK1/2 is a critical intermediary of PKC-mediated S6K1 activation. In the case of insulin- and forskolin-stimulated S6K1 activation, U0126 treatment, unlike the C4B expression, caused a partial blocking of S6K1 phosphorylation. Therefore, although MEK1/2 is an important downstream effector of c-Raf, other pathways that mediate S6K1 activation seem to rely on the basal activity of MEK1/2. In this context, the basal MEK1/2 activity has been shown to be important for insulin (72, 73)- and epidermal growth factor (71)-stimulated S6K1 activation. Overall, our study demonstrates that activation of MEK1/2 alone can be sufficient for the mTOR and S6K1 phosphorylation at specific sites in adult cardiocytes, suggesting that MEK1/2 is a critical player functioning downstream of c-Raf during PKC-mediated S6K1 activation. In support of this, previous studies demonstrate that c-Raf is sufficient to activate S6K1 in CCL39 cells (37) and that MEK1/2 is important for the S6K1 activation in HEK293E cells (71). In adult cardiocytes, a recent study also demonstrates that MEK1/2 is important for the activation of S6K2, a structurally related kinase (39).

To identify downstream components of MEK1/2 signaling, we focused on MAPK. Our studies using MKP-3 adenovirus suggest that ERK1/2 is a potential downstream player for the PKC/c-Raf/MEK pathway leading to S6K1 activation. The TPA-stimulated changes, including the retarded electrophoretic mobility and phosphorylation at Thr-421/Ser-424 sites of S6K1, are blocked significantly in cells expressing MKP-3. However, in insulin- and forskolin-stimulated cells, the retarded electrophoretic mobility is not significantly affected. Analysis of the phosphorylation pattern of S6K1 demonstrates that MKP-3 significantly blocks both the Thr-421/Ser-424 and the Thr-389 phosphorylations when these cells are stimulated with TPA. There are at least two possibilities for the loss of Thr-389 phosphorylation during MKP-3 expression: (i) both the basal, as observed during forskolin stimulation, and the stimulated, as observed during TPA stimulation, levels of Thr-421/Ser-424 phosphorylation, which is critical for the subsequent Thr-389 phosphorylation, are abolished and/or (ii) the phosphorylation and activation of other potential intermediates, such as mTOR that controls the Thr-389 phosphorylation, is affected. Our data on the Ser-2448 phosphorylation of mTOR indicate that the TPA- but not insulin- or forskolin-stimulated phosphorylation is lost in MKP-3-expressing cells. This suggests that the mTOR phosphorylation at Ser-2448 site is mediated specifically via c-Raf/MEK/ERK pathway in TPA-stimulated cardiocytes. Taken together these data indicate that the TPA-stimulated phosphorylation of both S6K1 at Thr-421/Ser-424 and Thr-389 sites and mTOR at Ser-2448 site requires the c-Raf/MEK/ERK pathway and that this pathway does not appear to be critical during insulin and forskolin stimulation. In support of our observation, a recent study (66) demonstrates that ERK1/2 is complexed with p70S6K, suggesting the possibility that ERK1/2 is a direct S6K kinase for the phosphorylation of one or more Ser/Thr-Pro sites, such as the Thr-421/Ser-424 sites in the pseudosubstrate domain.

It is important to note that the MKP-3 expression, compared with the C4B expression, was performed using significantly lower concentration of adenovirus (2-10 *versus* 250 m.o.i., respectively). Although the same cytomegalovirus promoter drove both viral expressions, the cellular concentrations of such expressed proteins depend upon their turnover rates. Furthermore, their biological effect can vary depending upon their specific activity. Although MKP-3 has been shown to be a phosphatase specific to ERK1/2, a high level expression can result in nonspecific effects on other phosphorylated proteins, including S6K1. Therefore, we used appropriate viral concentrations to demonstrate the specific effects

of the expressed proteins on the TPA-stimulated S6K1 activation relative to the insulin- and forskolin-stimulated S6K1 activation. Thus by infecting cardiocytes at a relatively lower concentration of MKP-3 adenovirus (2 and 10 m.o.i.), we were able to demonstrate that the MKP-3 significantly blocked the TPA- but not the insulin- or forskolin-stimulated S6K1 activation. On the other hand, the dominant negative c-Raf (C4B) virus, at a relatively higher concentration (250 m.o.i.), also blocked specifically the TPA-stimulated S6K1 activation. These studies demonstrate that c-Raf is a major downstream player of the PKC-mediated S6K1 activation and that ERK1/2, which functions further down stream c-Raf, is also a critical player to the TPA-stimulated S6K1 activation, although ERK1/2, unlike c-Raf, may also contribute partially to the forskolin-stimulated S6K1 activation.

Our experiments using two types of SB compounds demonstrate that the S6K1 activation by all three stimulants is not affected appreciably during this drug treatment. Importantly, SB compounds did not block the TPA-stimulated Thr-421/Ser-424 phosphorylation and retarded electrophoretic mobility of S6K1. Furthermore, these compounds did not block mTOR phosphorylation at Ser-2448, although the reason for the partial loss of Thr-389 phosphorylation during all three types of stimulation is not clear at the present time. Therefore, although both ERK1/2 and p38 MAPK activity are sharply reduced in MKP-3-expressing cells, the loss of S6K1 activation and Thr-421/Ser-424 phosphorylation in these cells is mostly due to the loss of ERK1/2 activity. A similar study (37) performed using MKP-1 demonstrates that the loss of ERK1/2 activity does not affect S6K1 activation. It is not clear whether the differences are due to a spatial difference in the loss of ERK1/2 activity, because MKP-1 is a nuclear enzyme. Overall, our studies strongly indicate that ERK1/2 is a major downstream kinase, transferring the c-Raf/MEK signal to S6K1 activation. However, our study does not rule out the possibility that one or more kinases necessary for S6K1 activation are regulated directly by c-Raf and/or MEK.

Studies using transgenic mice demonstrate that PKCε, compared with other isoforms, contributes to hypertrophic growth in a significant manner (74). In pressure-overloaded myocardium, our earlier study (42) shows the activation of at least three PKC isoforms, which includes the PKC_{ε} isoform. Furthermore, stimulation of cardiocytes with hypertrophic agents, such as phenylephrine, results in the membrane localization of PKC_{ε} and contributes to the activation of the c-Raf/MEK/ERK pathway (75). A latent complex between PKC_{ε}, c-Raf, and Ras has been identified recently in other cell types (76). Based on these reports and our present findings, we propose the following model to describe our studies. We propose that the activation of a specific PKC isoform contributes significantly to the S6K1 activation in pressure-overloaded myocardium. c-Raf is the major effector molecule that connects the PKC signaling to MEK1/2 for the activation of S6K1. MEK1/2, via regulating ERK1/2, controls both the Thr-421/Ser424 phosphorylation of S6K1 as well as mTOR phosphorylation required for the Thr-389 phosphorylation of S6K1. All these phosphorylations are important for subsequent Thr-229 phosphorylation and kinase activation, as described previously (24).

Overall, we demonstrate that c-Raf and its downstream components, MEK1/2 and ERK1/2, contribute in a significant way to the PKC- but not to the PI3K or PKA-mediated S6K1 activation in adult feline cardiocytes. Because we observe activation of the PKC/c-Raf/ERK pathway in pressure-overloaded myocardium, this pathway might be critical for hypertrophic cardiac growth via S6K1 activation and ribosomal biogenesis.

ACKNOWLEDGEMENTS

We thank Mary Barnes for cell isolation and Dr. Donald R. Menick for the MKP-3 adenovirus. We also thank Drs. Shintaro S. Nemoto, Michael R. Zile, and George Cooper IV for their help with pressure overload models and overall support.

FOOTNOTES

* This work was supported by research funds from the Department of Veterans Affairs (VA Merit Review and Research Enhancement Award Program), by Program Project Grant HL-48788 from NHLBI, National Institutes of Health, by National Student Research Award GM08716 (to C. D. W.), and by an American Heart Association postdoctoral fellowship 0120540U (to B. S.).The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Published, JBC Papers in Press, April 8, 2002, DOI 10.1074/jbc.M200328200

ABBREVIATIONS

The abbreviations used are: 5'-TOP, 5'-terminal oligopyrimidine; UTR, untranslated region; S6K1, p70S6 kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase-1/2; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; mTOR, mammalian target of rapamycin; LV, left ventricle; RV, right ventricle; RVPO, right ventricular pressure-overload; MKP-3, MAPK phosphatase-3; m.o.i., multiplicity of infection; PI3K, phosphatidylinositol 3-OH-kinase; E-64, *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; BIM, bisindolylmaleimide-I; SAPK, stress-activated protein kinase; JNK, c-Jun N-terminal kinase; MEK, MAPK/ERK kinase.

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1. Chua, B. H., Russo, L. A., Gordon, E. E., Kleinhans, B. J., and Morgan, H. E. (1987) *Am. J. Physiol.* **252**, C323-C327[Abstract/Free Full Text]
2. Cooper, G. (1987) *Annu. Rev. Physiol.* **49**, 501-518[CrossRef][Medline] [Order article via Infotrieve]

3. Watson, P. A., Haneda, T., and Morgan, H. E. (1989) *Am. J. Physiol.* **256**, C1257-C1261 [Abstract/Free Full Text]
4. Morgan, H. E., and Beinlich, C. J. (1997) *Mol. Cell. Biochem.* **176**, 145-151[CrossRef][Medline] [Order article via Infotrieve]
5. Morgan, H. E., and Baker, K. M. (1991) *Circulation* **83**, 13-25[Free Full Text]
6. Meyuhas, O. (2000) *Eur. J. Biochem.* **267**, 6321-6330[Abstract/Free Full Text]
7. Price, D. J., Nemenoff, R. A., and Avruch, J. (1989) *J. Biol. Chem.* **264**, 13825-13833 [Abstract/Free Full Text]
8. Stewart, M. J., and Thomas, G. (1994) *Bioessays* **16**, 809-815[CrossRef][Medline] [Order article via Infotrieve]
9. Price, D. J., Grove, J. R., Calvo, V., Avruch, J., and Bierer, B. E. (1992) *Science* **257**, 973-977 [Abstract/Free Full Text]
10. Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) *Cell* **69**, 1227-1236[CrossRef][Medline] [Order article via Infotrieve]
11. von Manteuffel, S. R., Dennis, P. B., Pullen, N., Gingras, A. C., Sonenberg, N., and Thomas, G. (1997) *Mol. Cell. Biol.* **17**, 5426-5436[Abstract]
12. Jefferies, H. B., Reinhard, C., Kozma, S. C., and Thomas, G. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 4441-4445[Abstract/Free Full Text]
13. Jeno, P., Ballou, L. M., Novak-Hofer, I., and Thomas, G. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 406-410[Abstract/Free Full Text]
14. Reinhard, C., Thomas, G., and Kozma, S. C. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 4052-4056 [Abstract/Free Full Text]
15. Reinhard, C., Fernandez, A., Lamb, N. J., and Thomas, G. (1994) *EMBO J.* **13**, 1557-1565 [Medline] [Order article via Infotrieve]
16. Grove, J. R., Banerjee, P., Balasubramanyam, A., Coffer, P. J., Price, D. J., Avruch, J., and Woodgett, J. R. (1991) *Mol. Cell. Biol.* **11**, 5541-5550[Abstract/Free Full Text]
17. Lane, H. A., Fernandez, A., Lamb, N. J., and Thomas, G. (1993) *Nature* **363**, 170-172[CrossRef] [Medline] [Order article via Infotrieve]
18. Shima, H., Pende, M., Chen, Y., Fumagalli, S., Thomas, G., and Kozma, S. C. (1998) *EMBO J.* **17**, 6649-6659[CrossRef][Medline] [Order article via Infotrieve]
19. Gout, I., Minami, T., Hara, K., Tsujishita, Y., Filonenko, V., Waterfield, M. D., and Yonezawa, K. (1998) *J. Biol. Chem.* **273**, 30061-30064[Abstract/Free Full Text]
20. Lee-Fruman, K. K., Kuo, C. J., Lippincott, J., Terada, N., and Blenis, J. (1999) *Oncogene* **18**, 5108-5114[CrossRef][Medline] [Order article via Infotrieve]
21. Koh, H., Jee, K., Lee, B., Kim, J., Kim, D., Yun, Y. H., Kim, J. W., Choi, H. S., and Chung, J. (1999) *Oncogene* **18**, 5115-5119[CrossRef][Medline] [Order article via Infotrieve]
22. Pullen, N., Dennis, P. B., Andjelkovic, M., Dufner, A., Kozma, S. C., Hemmings, B. A., and Thomas, G. (1998) *Science* **279**, 707-710[Abstract/Free Full Text]
23. Volarevic, S., and Thomas, G. (2001) *Prog. Nucleic Acids Res. Mol. Biol.* **65**, 101-127[Medline] [Order article via Infotrieve]
24. Dufner, A., and Thomas, G. (1999) *Exp. Cell Res.* **253**, 100-109[CrossRef][Medline] [Order article via Infotrieve]
25. Avruch, J. (1998) *Mol. Cell. Biochem.* **182**, 31-48[CrossRef][Medline] [Order article via Infotrieve]
26. Han, J. W., Pearson, R. B., Dennis, P. B., and Thomas, G. (1995) *J. Biol. Chem.* **270**, 21396-21403 [Abstract/Free Full Text]

27. Weng, Q. P., Kozlowski, M., Belham, C., Zhang, A., Comb, M. J., and Avruch, J. (1998) *J. Biol. Chem.* **273**, 16621-16629[Abstract/Free Full Text]
28. Mahalingam, M., and Templeton, D. J. (1996) *Mol. Cell. Biol.* **16**, 405-413[Abstract]
29. Mukhopadhyay, N. K., Price, D. J., Kyriakis, J. M., Pelech, S., Sanghera, J., and Avruch, J. (1992) *J. Biol. Chem.* **267**, 3325-3335[Abstract/Free Full Text]
30. Erikson, R. L. (1991) *J. Biol. Chem.* **266**, 6007-6010[Free Full Text]
31. Chou, M. M., and Blenis, J. (1995) *Curr. Opin. Cell Biol.* **7**, 806-814[CrossRef][Medline] [Order article via Infotrieve]
32. Chung, J., Grammer, T. C., Lemon, K. P., Kazlauskas, A., and Blenis, J. (1994) *Nature* **370**, 71-75 [CrossRef][Medline] [Order article via Infotrieve]
33. Monfar, M., Lemon, K. P., Grammer, T. C., Cheatham, L., Chung, J., Vlahos, C. J., and Blenis, J. (1995) *Mol. Cell. Biol.* **15**, 326-337[Abstract]
34. Ming, X. F., Burgering, B. M., Wennstrom, S., Claesson-Welsh, L., Heldin, C. H., Bos, J. L., Kozma, S. C., and Thomas, G. (1994) *Nature* **371**, 426-429[CrossRef][Medline] [Order article via Infotrieve]
35. Cass, L. A., Summers, S. A., Prendergast, G. V., Backer, J. M., Birnbaum, M. J., and Meinkoth, J. L. (1999) *Mol. Cell. Biol.* **19**, 5882-5891[Abstract/Free Full Text]
36. Filippa, N., Sable, C. L., Filloux, C., Hemmings, B., and Van Obberghen, E. (1999) *Mol. Cell. Biol.* **19**, 4989-5000[Abstract/Free Full Text]
37. Lenormand, P., McMahon, M., and Pouyssegur, J. (1996) *J. Biol. Chem.* **271**, 15762-15768 [Abstract/Free Full Text]
38. Kumar, V., Pandey, P., Sabatini, D., Kumar, M., Majumder, P. K., Bharti, A., Carmichael, G., Kufe, D., and Kharbanda, S. (2000) *EMBO J.* **19**, 1087-1097[CrossRef][Medline] [Order article via Infotrieve]
39. Wang, L., Gout, I., and Proud, C. G. (2001) *J. Biol. Chem.* **276**, 32670-32677 [Abstract/Free Full Text]
40. Eguchi, S., Iwasaki, H., Ueno, H., Frank, G. D., Motley, E. D., Eguchi, K., Marumo, F., Hirata, Y., and Inagami, T. (1999) *J. Biol. Chem.* **274**, 36843-36851[Abstract/Free Full Text]
41. Papst, P. J., Sugiyama, H., Nagasawa, M., Lucas, J. J., Maller, J. L., and Terada, N. (1998) *J. Biol. Chem.* **273**, 15077-15084[Abstract/Free Full Text]
42. Laser, M., Kasi, V. S., Hamawaki, M., Cooper, G., Kerr, C. M., and Kuppuswamy, D. (1998) *J. Biol. Chem.* **273**, 24610-24619[Abstract/Free Full Text]
43. Rozich, J. D., Barnes, M. A., Schmid, P. G., Zile, M. R., McDermott, P. J., and Cooper, G. (1995) *J. Mol. Cell. Cardiol.* **27**, 485-499[Medline] [Order article via Infotrieve]
44. Cooper, G., and Satava, R. M. (1974) *J. Appl. Physiol.* **37**, 762-764[Free Full Text]
45. Cooper, G., Mercer, W. E., Hoober, J. K., Gordon, P. R., Kent, R. L., Lauva, I. K., and Marino, T. A. (1986) *Circ. Res.* **58**, 692-705[Abstract]
46. Bruder, J. T., Heidecker, G., and Rapp, U. R. (1992) *Genes Dev.* **6**, 545-556[Abstract]
47. He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 2509-2514[Abstract/Free Full Text]
48. Kirshenbaum, L. A., MacLellan, W. R., Mazur, W., French, B. A., and Schneider, M. D. (1993) *J. Clin. Invest.* **92**, 381-387[Medline] [Order article via Infotrieve]
49. Gerard, R. D., and Meidell, R. S. (1995) *DNA Cloning: A Practical Approach: Mammalian Systems* , pp. 285-306, IRL Press, Oxford
50. Kuppuswamy, D., Kerr, C., Narishige, T., Kasi, V. S., Menick, D. R., and Cooper, G. (1997) *J.*

Biol. Chem. **272**, 4500-4508[Abstract/Free Full Text]

51. Nave, B. T., Ouwens, M., Withers, D. J., Alessi, D. R., and Shepherd, P. R. (1999) *Biochem. J.* **344**, 427-431[CrossRef][Medline] [Order article via Infotrieve]
52. Stokoe, D., and McCormick, F. (1997) *EMBO J.* **16**, 2384-2396[CrossRef][Medline] [Order article via Infotrieve]
53. Muda, M., Boschert, U., Dickinson, R., Martinou, J. C., Martinou, I., Camps, M., Schlegel, W., and Arkinstall, S. (1996) *J. Biol. Chem.* **271**, 4319-4326[Abstract/Free Full Text]
54. Muda, M., Theodosiou, A., Rodrigues, N., Boschert, U., Camps, M., Gillieron, C., Davies, K., Ashworth, A., and Arkinstall, S. (1996) *J. Biol. Chem.* **271**, 27205-27208[Abstract/Free Full Text]
55. Terada, N., Patel, H. R., Takase, K., Kohno, K., Nairn, A. C., and Gelfand, E. W. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 11477-11481[Abstract/Free Full Text]
56. Senbonmatsu, T., Ichihara, S., Price, E., Jr., Gaffney, F. A., and Inagami, T. (2000) *J. Clin. Invest.* **106**, R25-R29[Medline] [Order article via Infotrieve]
57. Beretta, L., Gingras, A. C., Svitkin, Y. V., Hall, M. N., and Sonenberg, N. (1996) *EMBO J.* **15**, 658-664[Medline] [Order article via Infotrieve]
58. Naga Prasad, S. V., Esposito, G., Mao, L., Koch, W. J., and Rockman, H. A. (2000) *J. Biol. Chem.* **275**, 4693-4698[Abstract/Free Full Text]
59. Takano, H., Komuro, I., Zou, Y., Kudoh, S., Yamazaki, T., and Yazaki, Y. (1996) *FEBS Lett.* **379**, 255-259[CrossRef][Medline] [Order article via Infotrieve]
60. Sadoshima, J., Qiu, Z., Morgan, J. P., and Izumo, S. (1995) *Circ. Res.* **76**, 1-15 [Abstract/Free Full Text]
61. Yamazaki, T., Tobe, K., Hoh, E., Maemura, K., Kaida, T., Komuro, I., Tamemoto, H., Kadowaki, T., Nagai, R., and Yazaki, Y. (1993) *J. Biol. Chem.* **268**, 12069-12076[Abstract/Free Full Text]
62. Ferrari, S., Bannwarth, W., Morley, S. J., Totty, N. F., and Thomas, G. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 7282-7286[Abstract/Free Full Text]
63. Pearson, R. B., Dennis, P. B., Han, J. W., Williamson, N. A., Kozma, S. C., Wettenhall, R. E., and Thomas, G. (1995) *EMBO J.* **14**, 5279-5287[Medline] [Order article via Infotrieve]
64. Moser, B. A., Dennis, P. B., Pullen, N., Pearson, R. B., Williamson, N. A., Wettenhall, R. E., Kozma, S. C., and Thomas, G. (1997) *Mol. Cell. Biol.* **17**, 5648-5655[Abstract]
65. Wu, G., Toyokawa, T., Hahn, H., and Dorn, G. W., 2nd (2000) *J. Biol. Chem.* **275**, 29927-29930 [Abstract/Free Full Text]
66. Gu, M., Lynch, J., and Brecher, P. (2000) *J. Biol. Chem.* **275**, 11389-11396 [Abstract/Free Full Text]
67. Boknik, P., Heinroth-Hoffmann, I., Kirchhefer, U., Knapp, J., Linck, B., Luss, H., Muller, T., Schmitz, W., Brodde, O., and Neumann, J. (2001) *Cardiovasc. Res.* **51**, 717-728[CrossRef] [Medline] [Order article via Infotrieve]
68. Weng, Q. P., Andrabi, K., Klippel, A., Kozlowski, M. T., Williams, L. T., and Avruch, J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 5744-5748[Abstract/Free Full Text]
69. Ballou, L. M., Luther, H., and Thomas, G. (1991) *Nature* **349**, 348-350[CrossRef][Medline] [Order article via Infotrieve]
70. Weng, Q. P., Andrabi, K., Kozlowski, M. T., Grove, J. R., and Avruch, J. (1995) *Mol. Cell. Biol.* **15**, 2333-2340[Abstract]
71. Martin, K. A., Schalm, S. S., Romanelli, A., Keon, K. L., and Blenis, J. (2001) *J. Biol. Chem.* **276**, 7892-7898[Abstract/Free Full Text]
72. Scott, P. H., and Lawrence, J. C., Jr. (1997) *FEBS Lett.* **409**, 171-176[CrossRef][Medline] [Order article via Infotrieve]

73. Herbert, T. P., Kilhams, G. R., Batty, I. H., and Proud, C. G. (2000) *J. Biol. Chem.* **275**, 11249-11256[Abstract/Free Full Text]
74. Bogoyevitch, M. A., Parker, P. J., and Sugden, P. H. (1993) *Circ. Res.* **72**, 757-767[Abstract]
75. Puceat, M., and Vassort, G. (1996) *Mol. Cell. Biochem.* **157**, 65-72[Medline] [Order article via Infotrieve]
76. Hamilton, M., Liao, J., Cathcart, M. K., and Wolfman, A. (2001) *J. Biol. Chem.* **276**, 29079-29090 [Abstract/Free Full Text]

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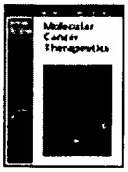

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Blood, January 15, 2006; 107(2): 642 - 650.
[Abstract] [Full Text] [PDF]

Symbol	Name	Synonyms	Organism
MAP3K1	mitogen-activated protein kinase kinase kinase 1	MAPK/ERK kinase kinase 1, MAPKKK1, MEKK, MEKK1, MEKK 1, MEK kinase 1, Mitogen-activated protein kinase kinase kinase 1	Homo sapiens

UniProt	Q13233
IntAct	Q13233
OMIM	600982
NCBI Gene	4214
NCBI RefSeq	XP_042066
NCBI RefSeq	XM_042066
NCBI UniGene	4214
NCBI Accession	AF042838, BX537527

Homologues of MAP3K1 ... new

Interaction information for this gene ...

Enhanced PubMed/Google query ... new

Over-expressed **MEKK1** co-immunoprecipitated with **alpha-actinin** in cell lysates.

MEKK1 interacts with **alpha-actinin** and localizes to stress fibers and focal adhesions.

These results provide strong evidence for the interaction between **MEKK1** and **alpha-actinin**.

Both endogenous and over-expressed **MEKK1** colocalized with **alpha-actinin** along **actin** stress fibers and at focal adhesions.

The results define the involvement of **MEKK1** in the induction of apoptosis by **genotoxins** but not **microtubule** altering drugs.

MEKK1 was required for maximal activation of ERK, JNK, and IKK, as well as for maximal **AP-1** and NF-kappaB transcriptional activities.

We conclude that Ras regulates TNF-alpha-induced chemokine expression by activating the **AP-1** pathway and enhancing transcriptional function of NF-kappaB, whereas **MEKK1** activates both the **AP-1** and NF-kappaB pathways.

Moreover, the **MEKK1** dominant negative mutant as well as deletion of the **AP-1** binding sites within the c-jun promoter inhibited the c-jun promoter activation by hypoxia.

Consistent with impaired osteoclastogenesis and reduced expression of TRAFs and **MEKK1**, we found that phosphorylation and activation of I kappa B, **NF-kappa B**, **ERKs**, and cJun/AP-1 are severely reduced in RANKL-treated TNFr1-null **osteoclast** precursors compared with wild type counterparts.

This compound also prevents activation of both IKKs and DNA binding of **NF-kappa B** induced by **MEKK** and NF-kappa B-inducing kinase.

A second **protein kinase** cascade leading to activation of the Jun kinases (JNKs) is dependent on **MEKK** (MEK kinase).

Eukaryotic cells respond to different extracellular stimuli by recruiting homologous signalling pathways that use members of the **MEKK**, MEK and ERK families of **protein kinases**.

Herein we demonstrate that full-length and cleaved **MEKK1 [?]** leads to permeability transition in the **mitochondria**.

Overall, this suggests that cleaved **MEKK1 [?]** leads to permeability transition contributing to MEKK1-induced apoptosis independent of cytochrome c release from the **mitochondria**.

Dominant negative **MEKK1** inhibits survival of **pancreatic cancer** cells.

Colony formation assays by transfection of dominant negative mutants of Ras, ERK or **MEKK1** into **pancreatic cancer** cell lines (BxPC-3, PANC-1, MIAPaCa-2 and AsPC-1) and an amnion-derived cell line (FL) revealed that DN-MEKK strongly inhibits the survival of colonies in **pancreatic cancer** cells, but

not in FL cells.

Recruitment of TRAFs and **MEKK1** ✩ leads to activation of downstream pathways, primarily I kappa B/NF-kappa B, **ERKs**, and cJun/AP-1.

Taxol treatment of cells, therefore, dissociates **MEKK1** ✩ activation from the regulation of the JNK pathway.

MEKK1 ✩ became activated in HEK293 cells exposed to **taxol**, but in contrast to etoposide-treatment, **taxol** failed to increase JNK activity.

Potentiation of apoptosis by low dose **stress** stimuli in cells expressing activated **MEK kinase 1** ✩.

The findings demonstrate that acute expression of an active form of **MEKK1** ✩ can potentiate the cell death response to external **stress** stimuli.

Cloning of rat **MEK kinase 1** ✩ **cDNA** reveals an endogenous membrane-associated 195-kDa protein with a large regulatory domain.

Instead, LPA induces the redistribution of **focal adhesion** kinase to **focal contact** regions of the cytoplasm membrane, and this event is abolished by pertussis toxin, dominant negative H-Ras, or dominant negative **MEKK1** ✩.

Mitogenic effect of orphan receptor TR3 and its regulation by **MEKK1** ✩ in **lung cancer** cells.

High **D-glucose** treatment induced **MEKK1** ✩ cleavage, whereas caspase inhibitors significantly attenuated the cleavage.

Role of receptor-interacting protein in **tumor necrosis factor-alpha** -dependent **MEKK1** ✩ activation.

Cisplatin-resistance involves the defective processing of **MEKK1** ✩ in human ovarian **adenocarcinoma** 2008/C13 cells.

Transcript stability was low in untreated **HeLa cells**, but increased in cells expressing a constitutively active form of the MAP kinase kinase kinase **MEKK1** ✩.

In cells exposed to genotoxic agents including **etoposide** and **cytosine arabinoside**, **MEKK1** ✩ is cleaved at Asp874 by caspases.

These findings suggest that **MEKK1** ✩ --> SEK1/MKK4 may function as an upstream kinase capable of activating both p38 MAPK and JNK/SAPK with subsequent induction of Cox-2 expression and **PGE2** production.

Activation of **MEKK1** ✩, which stimulates the JNK pathway, is not sufficient for **PC12 cell** differentiation but can induce apoptosis.

MAP/ERK kinase kinase 1 (**MEKK1** ✩), an upstream activator of MAP kinases, increases **E-selectin** promoter transcription and requires an intact PDII site for maximal induction.

The inducible acute expression at modest levels of the activated **MEKK1** ✩ kinase domain can be used to potentiate the apoptotic response to low dose ultraviolet irradiation and **cisplatin**.

Two **threonine** residues within this region in **Mekk1** ✩ at positions 560 and 572, but not the serine at 557, were shown to be essential for catalytic activity in this study.

Further examination of the **Mekk1** ✩ mutants isolated from 32P-labeled transfected **COS cells** showed that Thr-560 and Thr-572 were indeed phosphorylated after two-dimensional tryptic-chymotryptic phosphopeptide analysis.

Activation of **MEKK** ✩ by formyl-methionyl-leucyl-phenylalanine in human **neutrophils**. Mapping pathways for mitogen-activated protein kinase activation.

Molecular cloning of mitogen-activated protein/ERK kinase kinases (**MEKK** ✩) 2 and 3. Regulation of sequential phosphorylation pathways involving mitogen-activated protein kinase and c-Jun kinase.

Mapping of the MEK kinase gene (**Mekk [?]** ✩) to mouse chromosome 13 and human **chromosome 5**.

Expression of Galpha 13 (Q226L) induces P19 **stem cells** to primitive endoderm via **MEKK1 [?]** ✩, 2, or 4.

No significant effect on aggregation was seen when using selective inhibitors for p160(ROCK), **PKC**, or **MEKK1**.

By expressing a dominant-active form of **mitogen-activated protein kinase kinase kinase 1**, by exposure to tumor necrosis factor alpha, or by overexpression of p50/p65, we show that **NF-kappa B**

activates a transcription regulatory element of the **prostate-specific antigen** (PSA)-encoding gene, a marker for **prostate cancer** development, treatment, and progression.

MEKK1 expression is elevated 3-fold in mitosis and **microtubule** toxin-treated cells accumulated at G2/M of the cell cycle.

Mitogen-activated protein kinase kinase kinase 1 activates androgen receptor-dependent transcription and apoptosis in **prostate cancer**.

The results show that **MEKK1** is required for JNK activation in response to **microtubule** but not actin fiber toxins in embryonic stem cells.

Our findings demonstrate that expression of constitutively active **MEKK1** induces apoptosis in androgen receptor-positive but not in androgen receptor-negative **prostate cancer** cells.

These studies demonstrate an unanticipated link between **MEKK1** and hormone receptor signaling and have implications for the molecular basis of hormone-independent **prostate cancer** growth.

MEKK1 activation can protect cells from apoptosis in response to change in the integrity of the **microtubule cytoskeleton**.

These findings demonstrate that **MEKK1** contributes to the apoptotic response to **genotoxins**.

Following activation by **genotoxins**, **MEKK1** was cleaved in a caspase-dependent manner into an active 91-kDa kinase fragment.

The regulation of **MEKK1** by **genotoxins** involves its activation, which may be part of survival pathways, followed by its cleavage, which generates a proapoptotic kinase fragment able to activate caspases.

Protein kinase C phosphorylation may provide one mechanism for activating **MEKK**.

The N-terminal noncatalytic domain of **MEKK**, which contains several consensus **protein kinase C** phosphorylation sites, may, therefore, function as a negative regulatory domain.

Mammalian mitogen-activated **protein kinase** kinase kinase (**MEKK**) can function in a yeast mitogen-activated **protein kinase** pathway downstream of **protein kinase C**.

MEKK1 strongly stimulated recruitment of polyQ **polypeptides** into the particulate fraction.

Intracellular aggregation of **polypeptides** with expanded polyglutamine domain is stimulated by stress-activated kinase **MEKK1**.

MEKK1 also stimulated formation of IBs with two abnormal **polypeptides** lacking the polyQ domain, indicating that this kinase has a general effect on protein aggregation.

The exon IV-BDNF promoter activity was increased by **transfection** with CaM KII, **MEKK** and PKA, but not by CaM KIV.

Effects of constitutively active mutants of CaM KIV, MAPK kinase kinase (**MEKK**) and **protein kinase A** (PKA) on the exon IV-BDNF promoter activity were also evaluated by **transfection** and luciferase assay.

While active **MEKK1** was a potent activator of hypertrophic gene expression, this kinase did not induce **actin** organization and prevented phenylephrine-induced organization.

A Drosophila melanogaster **cDNA**, encoding a protein with sequence similarity to the **MEKK [?]** family of Ser/Thr kinases, was isolated from an eye-antennal imaginal disc **cDNA** library using a PCR-based approach.

Here, we describe the use of virus-induced gene silencing (VIGS) to study the role of candidate plant **MAP kinase kinase kinase** (MAPKKK) homologs of human **MEKK1** in pathogen-resistance pathways.

Calcitriol induces poly (adenosine diphosphate-ribose) polymerase cleavage, increases bax/bcl-2 ratio, reduces levels of phosphorylated mitogen-activated protein kinases (P-MAPKs; also known as extracellular signal-related kinase [ERK] 1/2) and phosphorylated Akt, induces caspase-dependent mitogen-activated protein kinase kinase (MEK) cleavage and upregulation of **MEK kinase-1**, all potential markers of the apoptotic pathway.

Expression of the dominant negative form of **MEKK1** had no influence on the ability of **retinoic acid** to induce either JNK activation or primitive **endoderm** formation in P19 stem cells.

Using a Jurkat T cell line transfected with dominant active (DA)-mitogen-activated protein kinase kinase kinase (**MEKK1**) in a tetracycline-regulated expression system, we found that expression of DA-MEKK1 results in the apoptosis of **Jurkat cells** in parallel with prolonged JNK activation.

In Swiss 3T3 and REF52 fibroblasts, activated **MEKK** induces cell death involving cytoplasmic shrinkage, nuclear condensation, and **DNA** fragmentation characteristic of apoptosis.

MEK kinase (**MEKK**) is part of a family of **serine-threonine protein kinases** that phosphorylate and activate MEKs independently of Raf.

Symbol	Name	Synonym/ DB-reference	Organism
[redacted]		**Life cycles of successful g**	
MAP3K1	mitogen-activated protein kinase kinase kinase 1	**MEKK**	Homo sapiens
MAP3K5	mitogen-activated protein kinase kinase kinase 5	**MEKK**5	Homo sapiens
MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	**MEKK**K 4	Homo sapiens
MAP3K4	mitogen-activated protein kinase kinase kinase 4	**MEKK**4	Homo sapiens
MAP3K2	mitogen-activated protein kinase kinase kinase 2	**MEKK**2	Homo sapiens
MAP3K3	mitogen-activated protein kinase kinase kinase 3	**MEKK**3	Homo sapiens
MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1	**MEKK**K 1	Homo sapiens
MAP4K2	mitogen-activated protein kinase kinase kinase kinase 2	**MEKK**K 2	Homo sapiens
MAP4K5	mitogen-activated protein kinase kinase kinase kinase 5	**MEKK**K 5	Homo sapiens
MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3	**MEKK**K 3	Homo sapiens
MINK1	misshapen-like kinase 1 (zebrafish)	**MEKK**K 6	Homo sapiens

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Terms	Documents
L5 and (jnk or mapk or sapk)	151

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L6	L5 and (jnk or mapk or sapk)	151	L6
L5	L1 and microinject$	186	L5
L4	L1 amd microinject$	0	L4
L3	L2 and (regulat$ with apoptosis with mekk1)	20	L3
L2	L1 and apoptosis	347	L2
L1	mekk1 or map4k1 or map-4-k-1 or map 4 k1 or hematopoietic progenitor kinase or hptk1 or hpk-1 or hpk1 or mapk/erk kinase kinase kinase or mek kinase kinase 1 or mekkk 1	497	L1

END OF SEARCH HISTORY

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Search	Most Recent Queries	Time	Result
#8	Search **(cell(AND (death) AND ("Etoposide" [MH])**	06:33:55	970
#7	Search **(cell and death) AND ("Etoposide" [MH])**	06:17:48	970
#5	Search **(genotoxin) AND ("Mutagens" [MH])**	06:15:20	97
#4	Search **(mekk) AND ("Mutagens" [MH])**	06:13:18	0
#1	Search **(ras) AND ("MAP4K1" [TIAB] OR "MAP-4-K-1" [TIAB] OR "MAP 4 K 1" [TIAB] OR "Hematopoietic progenitor kinase" [TIAB] OR "HPK1" [TIAB] OR "HPK-1" [TIAB] OR "HPK 1" [TIAB] OR "MAPK/ERK kinase kinase kinase 1" [TIAB] OR "MEK kinase kinase 1" [TIAB] OR "MEKKK 1" [TIAB] OR "Mitogen-activated protein kinase kinase kinase kinase 1" [TIAB])**	06:07:22	2

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- To combine searches use #search, e.g., #2 AND #3 or click query # for more options.

Search	Most Recent Queries	Time	Result
#15	Search **(cell death) AND ("Cytarabine" [MH])**	06:35:04	430
#8	Search **(cell(AND (death) AND ("Etoposide" [MH])**	06:33:55	970
#7	Search **(cell and death) AND ("Etoposide" [MH])**	06:17:48	970
#5	Search **(genotoxin) AND ("Mutagens" [MH])**	06:15:20	97
#4	Search **(mekk) AND ("Mutagens" [MH])**	06:13:18	0
#1	Search **(ras) AND ("MAP4K1" [TIAB] OR "MAP-4-K-1" [TIAB] OR "MAP 4 K 1" [TIAB] OR "Hematopoietic progenitor kinase" [TIAB] OR "HPK1" [TIAB] OR "HPK-1" [TIAB] OR "HPK 1" [TIAB] OR "MAPK/ERK kinase kinase kinase 1" [TIAB] OR "MEK kinase kinase 1" [TIAB] OR "MEKKK 1" [TIAB] OR "Mitogen-activated protein kinase kinase kinase kinase 1" [TIAB])**	06:07:22	2

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Search	Most Recent Queries	Time	Result
#48	**Related Articles** for **PubMed** (Select **16297711**)	07:58:04	361
#41	Search **(beauvericin) and (apoptosis)**	07:56:33	18
#46	Search **(beauvericin) and (apoptosis) and (mechanism)**	07:55:35	5
#45	Search **(beauvericin) and (apoptosis) and (ras)**	07:55:03	0
#44	Search **(beauvericin) and (apoptosis) and (mekk1)**	07:54:55	0
#43	Search **(beauvericin) and (apoptosis) and (mekk)**	07:54:39	0
#38	Search **(ras) and (apoptosis)**	07:49:34	2313
#37	Search **ras and apoptosis**	07:48:27	2313
#35	**Related Articles** for **PubMed** (Select **7744823**)	07:47:32	80
#27	**Related Articles** for **PubMed** (Select **8073291**)	07:41:22	152
#19	Search **(ras) AND ("MAP3K1" [TIAB] OR "MAP-3-K-1" [TIAB] OR "MAP 3 K 1" [TIAB] OR "MAPK/ERK kinase kinase 1" [TIAB] OR "MAPKKK1" [TIAB] OR "MAPKKK-1" [TIAB] OR "MAPKKK 1" [TIAB] OR "MEKK" [TIAB] OR "MEKK" [TIAB] OR "MEKK1" [TIAB] OR "MEKK-1" [TIAB] OR "MEKK 1" [TIAB] OR "MEKK 1" [TIAB] OR "MEK kinase 1" [TIAB] OR "Mitogen-activated protein kinase kinase kinase 1" [TIAB])**	07:18:40	84
#18	Search **(mekk) AND ("Etoposide" [MH])**	06:37:33	1
#17	Search **(mekk) AND ("Cytarabine" [MH])**	06:37:20	0
#15	Search **(cell death) AND ("Cytarabine" [MH])**	06:35:04	430
#8	Search **(cell(AND (death) AND ("Etoposide" [MH])**	06:33:55	970
#7	Search **(cell and death) AND ("Etoposide" [MH])**	06:17:48	970
#5	Search **(genotoxin) AND ("Mutagens" [MH])**	06:15:20	97
#4	Search **(mekk) AND ("Mutagens" [MH])**	06:13:18	0
#1	Search **(ras) AND ("MAP4K1" [TIAB] OR "MAP-4-K-1" [TIAB] OR "MAP 4 K 1" [TIAB] OR "Hematopoietic progenitor kinase" [TIAB] OR**	06:07:22	2

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- Search numbers may not be continuous; all searches are represented.
- To save search indefinitely, click query # and select Save in My NCBI.
- To combine searches use #search, e.g., #2 AND #3 or click query # for more options.

Search	Most Recent Queries	Time	Result
#38	**Related Articles** for **PubMed** (Select **6089191**)	09:37:55	122
#34	**Related Articles** for **PubMed** (Select **6611509**)	09:36:22	322
#56	**Related Articles** for **PubMed** (Select **6147754**)	09:35:18	132
#40	**Related Articles** for **PubMed** (Select **6380758**)	09:17:36	106
#25	**Related Articles** for **PubMed** (Select **2202421**)	09:07:16	89
#23	Search **(#18) purification**	08:57:33	74
#21	**Related Articles** for **PubMed** (Select **17028781**)	08:55:41	101
#19	Search **isolated ras**	08:51:33	1988
#18	Search **(cloned) and (ras)**	08:50:56	758
#17	Search **(isolated) and (ras)**	08:49:56	1988
#14	Search **(apoptosis) and (isolated ras)**	08:47:51	80
#13	Search **(apoptosis) and (ras) and (induction) and (isolation)**	08:45:24	6
#12	Search **(apoptosis) and (ras) and (induction) and (purification)**	08:44:59	7
#5	Search **(apoptosis) and (ras) and (induction)**	08:44:22	471
#4	Search **(apoptosis) and (ras) and (isolation)**	08:38:24	28
#1	Search **(apoptosis) and (ras) and (incubation)**	08:35:57	32

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- To combine searches use #search, e.g., #2 AND #3 or click query # for more options.

Search	Most Recent Queries	Time	Result
#59	Search **((ras) AND ("TCEAL1" [TIAB] OR "TCEAL-1" [TIAB] OR "TCEAL 1" [TIAB] OR "p21" [TIAB] OR "p-21" [TIAB] OR "p 21" [TIAB] OR "P21" [TIAB] OR "P-21" [TIAB] OR "P 21" [TIAB] OR "pp21" [TIAB] OR "pp-21" [TIAB] OR "pp 21" [TIAB] OR "SIIR" [TIAB] OR "SIIR" [TIAB])) AND (#58)apoptosis**	09:43:36	229
#58	Search **(ras) AND ("TCEAL1" [TIAB] OR "TCEAL-1" [TIAB] OR "TCEAL 1" [TIAB] OR "p21" [TIAB] OR "p-21" [TIAB] OR "p 21" [TIAB] OR "P21" [TIAB] OR "P-21" [TIAB] OR "P 21" [TIAB] OR "pp21" [TIAB] OR "pp-21" [TIAB] OR "pp 21" [TIAB] OR "SIIR" [TIAB] OR "SIIR" [TIAB])**	09:40:18	2528
#38	**Related Articles** for **PubMed** (Select **6089191**)	09:37:55	122
#34	**Related Articles** for **PubMed** (Select **6611509**)	09:36:22	322
#56	**Related Articles** for **PubMed** (Select **6147754**)	09:35:18	132
#40	**Related Articles** for **PubMed** (Select **6380758**)	09:17:36	106
#25	**Related Articles** for **PubMed** (Select **2202421**)	09:07:16	89
#23	Search **(#18) purification**	08:57:33	74
#21	**Related Articles** for **PubMed** (Select **17028781**)	08:55:41	101
#19	Search **isolated ras**	08:51:33	1988
#18	Search **(cloned) and (ras)**	08:50:56	758
#17	Search **(isolated) and (ras)**	08:49:56	1988
#14	Search **(apoptosis) and (isolated ras)**	08:47:51	80
#13	Search **(apoptosis) and (ras) and (induction) and (isolation)**	08:45:24	6
#12	Search **(apoptosis) and (ras) and (induction) and (purification)**	08:44:59	7
#5	Search **(apoptosis) and (ras) and (induction)**	08:44:22	471
#4	Search **(apoptosis) and (ras) and (isolation)**	08:38:24	28
#1	Search **(apoptosis) and (ras) and (incubation)**	08:35:57	32

"HPK1" [TIAB] OR "HPK-1" [TIAB] OR "HPK 1" [TIAB] OR "MAPK/ERK kinase kinase kinase 1" [TIAB] OR "MEK kinase kinase 1" [TIAB] OR "MEKKK 1" [TIAB] OR "Mitogen-activated protein kinase kinase kinase kinase 1" [TIAB])

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☐ 1: McGrath JP, Capon DJ, Goeddel DV, Levinson AD. Related Articles, Links

Comparative biochemical properties of normal and activated human ras p21 protein.
Nature. 1984 Aug 23-29;310(5979):644-9.
PMID: 6147754 [PubMed - indexed for MEDLINE]

☐ 2: Colby WW, Hayflick JS, Clark SG, Levinson AD. Related Articles, Links

Biochemical characterization of polypeptides encoded by mutated human Ha-ras1 genes.
Mol Cell Biol. 1986 Feb;6(2):730-4.
PMID: 3537694 [PubMed - indexed for MEDLINE]

☐ 3: Lacal JC, Anderson PS, Aaronson SA. Related Articles, Links

Deletion mutants of Harvey ras p21 protein reveal the absolute requirement of at least two distant regions for GTP-binding and transforming activities.
EMBO J. 1986 Apr;5(4):679-87.
PMID: 3011420 [PubMed - indexed for MEDLINE]

☐ 4: Tucker J, Sczakiel G, Feuerstein J, John J, Goody RS, Wittinghofer A. Related Articles, Links

Expression of p21 proteins in Escherichia coli and stereochemistry of the nucleotide-binding site.
EMBO J. 1986 Jun;5(6):1351-8.
PMID: 3015600 [PubMed - indexed for MEDLINE]

☐ 5: Gibbs JB, Sigal IS, Poe M, Scolnick EM. Related Articles, Links

Intrinsic GTPase activity distinguishes normal and oncogenic ras p21 molecules.
Proc Natl Acad Sci U S A. 1984 Sep;81(18):5704-8.
PMID: 6148751 [PubMed - indexed for MEDLINE]

☐ 6: Trahey M, Milley RJ, Cole GE, Innis M, Paterson H, Marshall CJ, Hall A, McCormick F. Related Articles, Links

Biochemical and biological properties of the human N-ras p21 protein.
Mol Cell Biol. 1987 Jan;7(1):541-4.
PMID: 3550423 [PubMed - indexed for MEDLINE]

☐ 7: Lacal JC, Srivastava SK, Anderson PS, Aaronson SA. Related Articles, Links

Ras p21 proteins with high or low GTPase activity can efficiently

transform NIH/3T3 cells.
Cell. 1986 Feb 28;44(4):609-17.
PMID: 3004741 [PubMed - indexed for MEDLINE]

☐ 8: Sigal IS, Gibbs JB, D'Alonzo JS, Temeles GL, Wolanski BS, Socher SH, Scolnick EM. Related Articles, Links

Mutant ras-encoded proteins with altered nucleotide binding exert dominant biological effects.
Proc Natl Acad Sci U S A. 1986 Feb;83(4):952-6.
PMID: 3513168 [PubMed - indexed for MEDLINE]

☐ 9: Stein RB, Robinson PS, Scolnick EM. Related Articles, Links

Photoaffinity labeling with GTP of viral p21 ras protein expressed in Escherichia coli.
J Virol. 1984 May;50(2):343-51.
PMID: 6323735 [PubMed - indexed for MEDLINE]

☐ 10: Masters SB, Landis CA, Bourne HR. Related Articles, Links

GTPase-inhibiting mutations in the alpha subunit of Gs.
Adv Second Messenger Phosphoprotein Res. 1990;24:70-5. Review. No abstract available.
PMID: 2119660 [PubMed - indexed for MEDLINE]

☐ 11: Hattori S, Ulsh LS, Halliday K, Shih TY. Related Articles, Links

Biochemical properties of a highly purified v-rasH p21 protein overproduced in Escherichia coli and inhibition of its activities by a monoclonal antibody.
Mol Cell Biol. 1985 Jun;5(6):1449-55.
PMID: 3162096 [PubMed - indexed for MEDLINE]

☐ 12: Landino LM, Macdonald TL. Related Articles, Links

Inhibition of the GDP/GTP exchange reaction of ras p21 by aluminum ion.
J Inorg Biochem. 1997 May 1;66(2):99-102.
PMID: 9112760 [PubMed - indexed for MEDLINE]

☐ 13: John J, Frech M, Wittinghofer A. Related Articles, Links

Biochemical properties of Ha-ras encoded p21 mutants and mechanism of the autophosphorylation reaction.
J Biol Chem. 1988 Aug 25;263(24):11792-9.
PMID: 3042780 [PubMed - indexed for MEDLINE]

☐ 14: Der CJ, Finkel T, Cooper GM. Related Articles, Links

Biological and biochemical properties of human rasH genes mutated at codon 61.
Cell. 1986 Jan 17;44(1):167-76.
PMID: 3510078 [PubMed - indexed for MEDLINE]

☐ 15: Giglione C, Parrini MC, Baouz S, Bernardi A, Parmeggiani A. Related Articles, Links

A new function of p120-GTPase-activating protein. Prevention of the guanine nucleotide exchange factor-stimulated nucleotide exchange on the active form of Ha-ras p21.
J Biol Chem. 1997 Oct 3;272(40):25128-34.
PMID: 9312123 [PubMed - indexed for MEDLINE]

☐ **16:** McCormick F. Related Articles, Links

GTP-binding proteins as oncogenes in human tumors.
Environ Health Perspect. 1991 Jun;93:17-8. Review. No abstract available.
PMID: 1773789 [PubMed - indexed for MEDLINE]

☐ **17:** Beckner SK, Hattori S, Shih TY. Related Articles, Links

The ras oncogene product p21 is not a regulatory component of adenylate cyclase.
Nature. 1985 Sep 5-11;317(6032):71-2.
PMID: 3929144 [PubMed - indexed for MEDLINE]

☐ **18:** Chung HH, Kim R, Kim SH. Related Articles, Links

Biochemical and biological activity of phosphorylated and non-phosphorylated ras p21 mutants.
Biochim Biophys Acta. 1992 Feb 11;1129(3):278-86.
PMID: 1536879 [PubMed - indexed for MEDLINE]

☐ **19:** Srivastava SK, Lacal JC, Reynolds SH, Aaronson SA. Related Articles, Links

Antibody of predetermined specificity to a carboxy-terminal region of H-ras gene products inhibits their guanine nucleotide-binding function.
Mol Cell Biol. 1985 Nov;5(11):3316-9.
PMID: 3915772 [PubMed - indexed for MEDLINE]

☐ **20:** Der CJ, Pan BT, Cooper GM. Related Articles, Links

rasH mutants deficient in GTP binding.
Mol Cell Biol. 1986 Sep;6(9):3291-4.
PMID: 3097518 [PubMed - indexed for MEDLINE]

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Symbol	Name	Synonym/ DB-reference	Organism
[redacted]		**Life cycles of successful g**	
RASA1	**RAS** p21 protein activator (GTPase activating protein) 1		Homo sapiens
K**RAS**	v-Ki-**ras**2 Kirsten rat sarcoma viral oncogene homolog		Homo sapiens
R**RAS**	related **RAS** viral (r-ras) oncogene homolog		Homo sapiens
H**RAS**	v-Ha-**ras** Harvey rat sarcoma viral oncogene homolog		Homo sapiens
RASGRF1	**Ras** protein-specific guanine nucleotide-releasing factor 1		Homo sapiens
RASA2	**RAS** p21 protein activator 2		Homo sapiens
RASGRF2	**Ras** protein-specific guanine nucleotide-releasing factor 2		Homo sapiens
RASAL1	**RAS** protein activator like 1 (GAP1 like)		Homo sapiens
RAB5A	RAB5A, member **RAS** oncogene family		Homo sapiens
RAB3A	RAB3A, member **RAS** oncogene family		Homo sapiens
RAC2	**ras**-related C3 botulinum toxin substrate 2 (rho family, small GTP binding pro...		Homo sapiens
RAB4A	RAB4A, member **RAS** oncogene family		Homo sapiens
IQGAP1	IQ motif containing GTPase activating protein 1	**Ras** GTPase-activating-like protein IQGAP1	Homo sapiens
RAB7	RAB7, member **RAS** oncogene family		Homo sapiens
RALA	v-ral simian leukemia viral oncogene homolog A (**ras** related)		Homo sapiens
RAB6A	RAB6A, member **RAS** oncogene family		Homo sapiens
RAB2	RAB2, member **RAS** oncogene family		Homo sapiens
RAB8A	RAB8A, member **RAS** oncogene family		Homo sapiens
RAB27A	RAB27A, member **RAS** oncogene family		Homo sapiens
RAP2A	RAP2A, member of **RAS** oncogene family		Homo sapiens
RAP1B	RAP1B, member of **RAS** oncogene family		Homo sapiens
RRAD	**Ras**-related associated with diabetes		Homo sapiens
RAC3	**ras**-related C3 botulinum toxin substrate 3 (rho family, small GTP binding pro...		Homo sapiens
RAB9A	RAB9A, member **RAS** oncogene family		Homo sapiens
MMP19	matrix metallopeptidase 19	**RASI**-1	Homo sapiens
PLA2G2A	phospholipase A2, group IIA (platelets, synovial fluid)	**RAS**F-A	Homo sapiens
RHEB	**Ras** homolog enriched in brain		Homo sapiens
RAB3B	RAB3B, member **RAS** oncogene family		Homo sapiens

RAP2B	RAP2B, member of **RAS** oncogene family		Homo sapiens
RHEBP1	**Ras**-homolog enriched in brain pseudogene 1		Homo sapiens
RIT2	**Ras**-like without CAAX 2		Homo sapiens
RALB	v-ral simian leukemia viral oncogene homolog B (**ras** related; GTP binding prot...		Homo sapiens
RSU1	**Ras** suppressor protein 1		Homo sapiens
RAB5B	RAB5B, member **RAS** oncogene family		Homo sapiens
RAB7L1	RAB7, member **RAS** oncogene family-like 1		Homo sapiens
RIT1	**Ras**-like without CAAX 1		Homo sapiens
RGL2	ral guanine nucleotide dissociation stimulator-like 2	**RAS**-associated protein RAB2L	Homo sapiens
RAB11B	RAB11B, member **RAS** oncogene family		Homo sapiens
RAB13	RAB13, member **RAS** oncogene family		Homo sapiens
SYNGAP1	synaptic **Ras** GTPase activating protein 1 homolog (rat)		Homo sapiens
RAB27B	RAB27B, member **RAS** oncogene family		Homo sapiens
RAB5C	RAB5C, member **RAS** oncogene family		Homo sapiens
RREB1	**ras** responsive element binding protein 1		Homo sapiens
RABIF	RAB interacting factor	**RAS**GFR3	Homo sapiens
SHOC2	soc-2 suppressor of clear homolog (C. elegans)	**Ras**-binding protein Sur-8	Homo sapiens
RAB33A	RAB33A, member **RAS** oncogene family		Homo sapiens
GEM	GTP binding protein overexpressed in skeletal muscle	**RAS**-like protein KIR	Homo sapiens
RAB28	RAB28, member **RAS** oncogene family		Homo sapiens
FNTA	farnesyltransfe**ras**e, CAAX box, alpha		Homo sapiens
FNTB	farnesyltransfe**ras**e, CAAX box, beta		Homo sapiens

List truncated. Use quotes for exact search, e.g. "SNF1"

Involvement of hematopoietic progenitor kinase 1 in T cell receptor signaling.

Hematopoietic progenitor kinase 1 (HPK1), a mammalian Ste20-related serine/threonine protein kinase, is a hematopoietic-specific upstream activator of the c-Jun N-terminal kinase. Here, we provide evidence to demonstrate the involvement of HPK1 in T cell receptor (TCR) signaling. HPK1 was activated and tyrosine-phosphorylated with similar kinetics following TCR/CD3 or pervanadate stimulation. Co-expression of protein-tyrosine kinases, Lck and Zap70, with HPK1 led to HPK1 activation and tyrosine phosphorylation in transfected mammalian cells. Upon TCR/CD3 stimulation, HPK1 formed inducible complexes with the adapters Nck and Crk with different kinetics, whereas it constitutively interacted with the adapters Grb2 and CrkL in Jurkat T cells. Interestingly, HPK1 also inducibly associated with linker for activation of T cells (LAT) through its proline-rich motif and translocated into glycolipid-enriched microdomains (also called lipid rafts) following TCR/CD3 stimulation, suggesting a critical role for LAT in the regulation of HPK1. Together, these results identify HPK1 as a new component of TCR signaling. T cell-specific signaling molecules Lck, Zap70, and LAT play roles in the regulation of HPK1 during TCR signaling. Differential complex formation between HPK1 and adapters highlights the possible involvement of HPK1 in multiple signaling pathways in T cells.

J Biol Chem (2001-06-01)

PMID: 11279207

PubMed

Fulltext - Related articles

Symbol	Name	Synonym/ DB-reference	Organism
[redacted]		Life cycles of successful g	
MAP3K1	mitogen-activated protein kinase kinase kinase 1	**MEKK**	Homo sapiens
MAP3K5	mitogen-activated protein kinase kinase kinase 5	**MEKK**5	Homo sapiens
MAP4K4	mitogen-activated protein kinase kinase kinase kinase 4	**MEKK**K 4	Homo sapiens
MAP3K4	mitogen-activated protein kinase kinase kinase 4	**MEKK**4	Homo sapiens
MAP3K2	mitogen-activated protein kinase kinase kinase 2	**MEKK**2	Homo sapiens
MAP3K3	mitogen-activated protein kinase kinase kinase 3	**MEKK**3	Homo sapiens
MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1	**MEKK**K 1	Homo sapiens
MAP4K2	mitogen-activated protein kinase kinase kinase kinase 2	**MEKK**K 2	Homo sapiens
MAP4K5	mitogen-activated protein kinase kinase kinase kinase 5	**MEKK**K 5	Homo sapiens
MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3	**MEKK**K 3	Homo sapiens
MINK1	misshapen-like kinase 1 (zebrafish)	**MEKK**K 6	Homo sapiens

1: Int J Cancer. 1995 May 16;61(4):502-8.

Links

MCF-7 human mammary adenocarcinoma cell death in vitro in response to hormone-withdrawal and DNA damage.

Wilson JW, **Wakeling AE**, **Morris ID**, **Hickman JA**, **Dive C**.

Molecular Pharmacology Group, School of Biological Sciences, University of Manchester, UK.

Anti-oestrogens exert a tumoristatic effect on estrogen-receptor-positive breast carcinomas in vivo. At a cellular level this may reflect inhibition of cell proliferation and/or cell death counterbalanced by continued proliferation of a cell subpopulation. We evaluated the MCF-7 human mammary adenocarcinoma cell line as an in vitro model to study the effects of the novel oestrogen antagonist ICI 182,720 on cell population dynamics (cell gain vs. cell loss). After oestrogen-withdrawal monolayer cell number declined over 10 days, accompanied by cell detachment. This decrease in viable cell number was elevated 2-fold by ICI 182,780. Detached cells exhibited DNA fragments of 50 and 300 kbp, typical of apoptotic cells. However, internucleosomal cleavage to 180 bp integer fragments was not seen, and these detached cells exhibited a morphology which was not consistent with apoptosis. The remaining attached monolayer cells were morphologically viable (> 99%) with regard to both nuclear morphology and plasma membrane integrity. There was no difference in cell cycle phase distribution between oestrogen-withdrawn and ICI 182,780-treated cells; both induced accumulation in G1 phase. MCF-7 cells were also exposed to a variety of DNA damaging agents known to induce apoptosis in other cell types. We could demonstrate only limited induction of morphologically recognisable apoptosis in MCF-7 cells treated with methyl methanesulphonate. Our results add to the controversy surrounding the ability of the MCF-7 cell line to undergo apoptosis in vitro in response to anti-oestrogen therapies.

PMID: 7759156 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1995 Jan 3;92(1):102-6.

Different topoisomerase II antitumor drugs direct similar specific long-range fragmentation of an amplified c-MYC gene locus in living cells and in high-salt-extracted nuclei.

Gromova II, **Thomsen B**, **Razin SV**.

Department of Molecular Biology, University of Aarhus, Denmark.

We have analyzed the long-range distribution of topoisomerase II-mediated cleavages induced in an amplified human c-MYC gene locus in the presence of several antitumor agents. The long-range cleavage patterns were found to be nonrandom and similar for all antitumor drugs tested. Cleavages occurred within several kilobase-long areas (approximately 5 kb) highly accessible to topoisomerase II and separated by extended regions (approximately 70-100 kb) of less accessibility, possibly reflecting the mode of DNA organization into loops along the chromosome. Within the cleavage areas, the patterns of cleavage sites showed a certain dependence on the type of drug used for entrapment of topoisomerase II-DNA complexes. Importantly, distribution of cleavage areas in native chromatin and histone-depleted nuclei was very similar, if not identical, suggesting that the primary target of antitumor agents in vivo is topoisomerase II associated with the high-salt-insoluble nuclear matrix. These data show that matrix-attached DNA is preferentially damaged by topoisomerase II-targeting agents, which may be an important cellular event contributing to drug-induced cell death.

PMID: 7816796 [PubMed - indexed for MEDLINE]

1: Biochimie. 1995;77(11):893-9.

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Induction of apoptotic cell death by DNA topoisomerase II inhibitors.

Negri C, **Bernardi R**, **Donzelli M**, **Scovassi AI**.

Istituto di Genetica Biochemica Evoluzionistica CNR, Pavia, Italy.

We have analyzed the interference of antitumoral drugs acting through the inhibition of DNA topoisomerase II on the human HeLa cell metabolism. Different compounds characterized by a diverse mechanism of action have been used, namely m-amsacrine, an intercalative drug, etoposide, which does not intercalate DNA, and suramin, which exerts its effect through an unknown mechanism. In HeLa cells treated with increasing doses of these drugs, we have examined cell viability and DNA synthesis capacity, and we have evaluated topoisomerase II activity. Cellular morphology and DNA integrity have been studied in order to characterize the mechanism of cell death. The results we have obtained clearly indicate that topoisomerase II poisons induce cell death by apoptosis. These observations suggest a role of the inhibition of topoisomerase II activity in the apoptotic program.

PMID: 8824770 [PubMed - indexed for MEDLINE]

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Roles of nonhomologous end-joining pathways in surviving topoisomerase II-mediated DNA damage. [Mol Cancer Ther. 2006]

Molecular effects of topoisomerase II inhibitors in AML cell lines: correlation of apoptosis with topoisomerase II activity but not with DNA damage. [Leukemia. 1999]

Cross-resistance of an amsacrine-resistant human leukemia line to topoisomerase II reactive DNA intercalating agents. Evidence for two topoisomerase II directed drug actions. [Biochemistry. 1991]

Human small cell lung cancer NYH cells selected for resistance to the bisdioxopiperazine topoisomerase II catalytic inhibitor ICRF-187 demonstrate a functional R162Q mutation in the Walker A consensus ATP binding domain of the alpha isoform. [Cancer Res. 1999]

Characterization of an amsacrine-resistant line of human leukemia cells. Evidence for a drug-resistant form of topoisomerase II. [J Biol Chem. 1989]

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Differential involvement of MEK kinase 1 (MEKK1) in the induction of apoptosis in response to microtubule-targeted drugs versus DNA damaging agents.

MEK kinase 1 (MEKK1) is a 196-kDa enzyme that is involved in the regulation of the c-Jun N-terminal kinase (JNK) pathway and apoptosis. In cells exposed to genotoxic agents including **etoposide** and **cytosine arabinoside**, MEKK1 is cleaved at Asp874 by caspases. The cleaved kinase domain of MEKK1, itself, stimulates caspase activity leading to apoptosis. Kinase-inactive MEKK1 expressed in HEK293 cells effectively blocks genotoxin-induced apoptosis. Treatment of cells with **taxol**, a **microtubule** stabilizing agent, did not induce MEKK1 cleavage in cells, and kinase-inactive MEKK1 expression failed to block taxol-induced apoptosis. MEKK1 became activated in HEK293 cells exposed to **taxol**, but in contrast to etoposide-treatment, **taxol** failed to increase JNK activity. **Taxol** treatment of cells, therefore, dissociates MEKK1 activation from the regulation of the JNK pathway. Overexpression of anti-apoptotic Bcl2 **blocked** MEKK1 and taxol-induced apoptosis but did not block the caspase-dependent cleavage of MEKK1 in response to **etoposide**. This indicates Bcl2 inhibition of apoptosis is, therefore, downstream of caspase-dependent MEKK1 cleavage. The results define the involvement of MEKK1 in the induction of apoptosis by **genotoxins** but not **microtubule** altering drugs.

J Biol Chem (1999-04-16)

PMID: 10196170

PubMed

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1: Mol Pharmacol. 1994 Nov;46(5):890-5.

Programmed cell death (apoptosis) of mouse fibroblasts is induced by the topoisomerase II inhibitor etoposide.

Mizumoto K, **Rothman RJ**, **Farber JL**.

Department of Pathology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107.

The mechanism by which etoposide, a topoisomerase II inhibitor, killed replicating mouse L929 fibroblasts was investigated. Etoposide at 10 microM killed 70% of the cells within 4 days, a result that was accompanied by DNA fragmentation. A characteristic "ladder" pattern of DNA fragmentation was confirmed by agarose gel electrophoresis. Simultaneous exposure of the cells to 10 microM etoposide plus 1 microM cycloheximide reduced both the extent of cell killing and the fragmentation of DNA. Delayed addition of cycloheximide protected cells only if cycloheximide was added 1-6 hr after exposure to etoposide. When added 6-24 hr after treatment with etoposide, cycloheximide lost the ability to protect cells. Cell growth was completely inhibited by either etoposide or cycloheximide. Furthermore, DNA synthesis was inhibited by either etoposide or cycloheximide within 6 hr. Protein synthesis, however, was not inhibited by etoposide. Thus, the ability of cycloheximide to protect cells correlated with inhibition of protein synthesis, rather than inhibition of DNA synthesis. A 1-hr exposure to 2.5 mM N-methyl-N-nitrosourea similarly inhibited DNA synthesis within 6 hr. without affecting protein synthesis. However, no loss of viability accompanied N-methyl-N-nitrosourea treatment. Thus, an imbalance between protein synthesis and DNA synthesis cannot explain the cell killing by etoposide. H-7, a protein kinase C inhibitor, prevented the cell killing and DNA fragmentation, whereas aurintricarboxylic acid, an endonuclease inhibitor, reduced the extent of DNA fragmentation but did not have an effect on cell killing. The data document that the killing of replicating mouse fibroblasts by etoposide represents an example of programmed cell death (apoptosis) that depends on protein synthesis. Although protein synthesis is required during the first 24 hr of exposure to etoposide, cell death is delayed until several days later.

PMID: 7969076 [PubMed - indexed for MEDLINE]

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Differential involvement of MEK kinase 1 (MEKK1) in the induction of apoptosis in response to microtubule-targeted drugs versus DNA damaging agents.

MEK kinase 1 (MEKK1) is a 196-kDa enzyme that is involved in the regulation of the c-Jun N-terminal kinase (JNK) pathway and apoptosis. In cells exposed to genotoxic agents including etoposide and cytosine arabinoside, MEKK1 is cleaved at Asp874 by caspases. The cleaved kinase domain of MEKK1, itself, stimulates caspase activity leading to apoptosis. Kinase-inactive MEKK1 expressed in HEK293 cells effectively blocks genotoxin-induced apoptosis. Treatment of cells with taxol, a microtubule stabilizing agent, did not induce MEKK1 cleavage in cells, and kinase-inactive MEKK1 expression failed to block taxol-induced apoptosis. MEKK1 became activated in HEK293 cells exposed to taxol, but in contrast to etoposide-treatment, taxol failed to increase JNK activity. Taxol treatment of cells, therefore, dissociates MEKK1 activation from the regulation of the JNK pathway. Overexpression of anti-apoptotic Bcl2 blocked MEKK1 and taxol-induced apoptosis but did not block the caspase-dependent cleavage of MEKK1 in response to etoposide. This indicates Bcl2 inhibition of apoptosis is, therefore, downstream of caspase-dependent MEKK1 cleavage. The results define the involvement of MEKK1 in the induction of apoptosis by genotoxins but not microtubule altering drugs.

J Biol Chem (1999-04-16)

PMID: 10196170

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Symbol	Name	Synonyms	Organism
MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1	Hematopoietic progenitor kinase, HPK1, MAPK/ERK kinase kinase kinase 1, MEK kinase kinase 1, MEKKK 1, Mitogen-activated protein kinase kinase kinase kinase 1	Homo sapiens

UniProt	Q92918
IntAct	Q92918
OMIM	601983
NCBI Gene	11184
NCBI RefSeq	NP_009112
NCBI RefSeq	NM_007181
NCBI UniGene	11184
NCBI Accession	Q92918, U66464

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Cleavage of **HPK1** was blocked by peptide inhibitors for **caspases**.

By testing **HPK1** proteins with in vivo and in vitro cleavage assays, we showed that aspartic acid residue 385 is the target for **caspases**.

In order to further characterize the HPK1-mediated JNK signaling cascade, we searched for HPK1-interacting **proteins** that could regulate **HPK1**.

SH2/SH3 adaptor **proteins** can link tyrosine kinases to a Ste20-related protein kinase, **HPK1**.

Here, we provide evidence to demonstrate the involvement of **HPK1** in **T cell receptor** (TCR) signaling.

We report here that exposure of hematopoietic Jurkat **T cells** to genotoxic agents is associated with activation of **HPK1**.

We have cloned a novel **protein kinase**, termed hematopoietic progenitor kinase 1 (**HPK1**), that is expressed predominantly in hematopoietic cells, including early progenitor cells.

Using isothermal titration **calorimetry** and **x-ray crystallography**, the binding of the **HPK1 [?]** motif to Mona/Gads SH3C has now been characterized in molecular detail.

1: Haematologica. 2006 Jul;91(7):912-9.

haematologica the hematology journal Links

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Haematologica. 2006 Jul;91(7):868.

The mechanisms of Ara-C-induced apoptosis of resting B-chronic lymphocytic leukemia cells.

de Vries JF, **Falkenburg JH**, **Willemze R**, **Barge RM**.

Department of Hematology, Leiden University Medical Center, P.O. Box 9600 2300 RC Leiden, The Netherlands. j.f.vries@lumc.nl

BACKGROUND AND OBJECTIVES: Cytarabine (Ara-C) is commonly used for the treatment of acute leukemia. Incorporation of Ara-C into DNA is a key event in the mechanism of killing of proliferating leukemic cells. Previously, we demonstrated that Ara-C was cytotoxic to proliferating but not to resting (G(O)) malignant cells from patients with acute leukemia. In contrast, here we show unexpected apoptosis of G(O) B-chronic lymphocytic leukemia (CLL) cells by Ara-C in a dose-dependent manner. In this study we analyzed which cellular processes were involved in Ara-C-mediated killing of G(O)-B-CLL cells. DESIGN AND METHODS: Using primary B-CLL cells (>98% in G(O)), we examined the mechanisms of Ara-C-mediated apoptosis in resting G(O) cells. CFSE-based cytotoxicity assays combined with cell cycle analysis were used to perform a long-term analysis of Ara-C-mediated killing of B-CLL cells. The effects of Ara-C on DNA and RNA synthesis were studied using various 3H-incorporation experiments. RESULTS: Ara-C-mediated cell death of B-CLL cells showed the characteristics of normal apoptosis, such as phosphatidyl serine exposure and caspase activation. The mechanism of killing of quiescent B-CLL cells by Ara-C was shown not to be dependent on DNA replication. In contrast, CD40L-activated B-CLL cells showed S-phase-specific depletion of proliferating CLL cells. We demonstrated that Ara-C was converted into its active triphosphate by G(O)-B-CLL cells, coinciding with a 30% inhibition of RNA synthesis. INTERPRETATION AND CONCLUSIONS: In conclusion, our data indicate that Ara-C can induce apoptosis in resting G(O)-B-CLL cells using a mechanism independent of cell proliferation and DNA replication but associated with inhibition of RNA synthesis and downregulation of Mcl-1.

PMID: 16818278 [PubMed - indexed for MEDLINE]

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1: Exp Cell Res. 2003 Feb 1;283(1):80-90.

The kinase domain of MEKK1 induces apoptosis by dysregulation of MAP kinase pathways.

Boldt S, **Weidle UH**, **Kolch W**.

Beatson Institute for Cancer Research, Cancer Research UK, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, UK. sboldt@beatson.gla.ac.uk

MAP kinase pathways comprise a group of parallel protein phosphorylation cascades, which are involved in signaling triggered by a variety of stimuli. Previous findings suggested that the ERK and the JNK pathways have opposing roles in regulating proliferation and survival or apoptosis and that apoptosis can be promoted by inhibiting the ERK pathway or by activation of the JNK pathway. In order to test this hypothesis and explore whether it can be exploited as a strategy for killing human cancer cells, we used gene transfer experiments with a range of cancer cell lines. We expressed the catalytic fragment of human MEKK1 to activate JNK and the Ras-binding domain (RBD) of Raf-1 to inhibit the Ras-ERK pathway. In addition, we designed several RBD-MEKK1 fusion proteins aiming to simultaneously activate the JNK and block the ERK pathway. We found that the MEKK1 proteins as well as the RBD alone could reduce colony formation in all cell lines. The survival time of MEKK1-expressing cells depended on the cell line. In HeLa cells, survival could be prolonged by inhibition of caspases but not by coexpression of the anti-apoptotic protein Bcl-2. Due to a lower kinase activity the RBD-MEKK1 fusion proteins were less effective in apoptosis induction than the MEKK1 kinase domain alone. Using mutant forms of Ras and Raf-1 we could show that the reduced kinase activity of RBD-MEKK1 fusion proteins was caused by binding to the Ras protein. The expression of lethal doses of MEKK1 resulted in a strong activation of all three major MAP kinase families JNK, ERK, and p38. Blocking these pathways either by coexpressing a dominant negative form of MKK4 or with inhibitors of MEK or p38 failed to inhibit apoptosis. This suggests that MEKK1 induces apoptosis by causing a general deregulation of MAP kinase signaling rather than by the activation of a single pathway. Copyright 2003 Elsevier Science (USA)

PMID: 12565821 [PubMed - indexed for MEDLINE]

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1: J Biol Chem. 2002 Jan 18;277(3):1828-36. Epub 2001 Nov 6.

Green tea polyphenol stimulates a Ras, MEKK1, MEK3, and p38 cascade to increase activator protein 1 factor-dependent involucrin gene expression in normal human keratinocytes.

Balasubramanian S, **Efimova T**, **Eckert RL**.

Department of Physiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4970, USA.

(-)-Epigallocatechin-3-gallate (EGCG) is an important bioactive constituent of green tea that efficiently reduces epidermal cancer cell proliferation. This inhibition is associated with a reduction in activator protein 1 (AP1) transcription factor level and activity. However, its effects on AP1 function in normal epidermal cells have not been extensively explored. Our present studies show that EGCG regulates normal keratinocyte function. To understand the mechanism of action, we examined the effects of EGCG on AP1 factor activity, MAPK signal transduction, and expression of the AP1 factor-regulated human involucrin (hINV) gene. EGCG increases hINV promoter activity in a concentration-dependent manner that requires the presence of an intact hINV promoter AP1 factor binding site. This response appears to be physiologic, as endogenous hINV gene expression is also increased. Fra-1, Fra-2, FosB, JunB, JunD, c-Jun, and c-Fos levels are increased by EGCG treatment, as is AP1 factor binding to hINV promoter AP1 site. Gel mobility shift studies show that this complex contains Fra-1 and JunD. Signal transduction analysis indicates that the EGCG response requires Ras, MEKK1, MEK3, and p38 kinases. Kinase assays and inhibitor studies suggest that p38delta is the p38 isoform responsible for the regulation. These changes are also associated with a cessation of cell proliferation and enhanced cornified envelope formation. These studies show that in normal human keratinocytes EGCG markedly increases, via a MAPK signaling mechanism, AP1 factor-associated responses.

PMID: 11698415 [PubMed - indexed for MEDLINE]

Oct 24 2006 07:33:51

1: Mol Reprod Dev. 1995 Dec;42(4):459-67.

Links

Transcriptional regulation by MAP kinases.

Davis RJ.

Department of Biochemistry and Molecular Biology, Howard Hughes Medical Institute, University of Massachusetts Medical Center, Worcester, MA 01605, USA.

Tyrosine kinase growth factor receptors activate MAP kinase by a complex mechanism involving the SH2/3 protein Grb2, the exchange protein Sos, and Ras. The GTP-bound Ras protein binds to the Raf kinase and initiates a protein kinase cascade that leads to MAP kinase activation. Three MAP kinase kinase kinases have been described--c-Raf, c-Mos, and Mekk--that phosphorylate and activate Mek, the MAP kinase kinase. Activated Mek phosphorylates and activates MAP kinase. Subsequently, the activated MAP kinase translocates into the nucleus where many of the physiological targets of the MAP kinase signal transduction pathway are located. These substrates include transcription factors that are regulated by MAP kinase phosphorylation (e.g., Elk-1, c-Myc, c-Jun, c-Fos, and C/EBP beta). Thus the MAP kinase pathway represents a significant mechanism of signal transduction by growth factor receptors from the cell surface to the nucleus that results in the regulation of gene expression. Three MAP kinase homologs have been identified in the rat: Erk1, Erk2, and Erk3. Human MAP kinases that are similar to the rat Erk kinases have also been identified by molecular cloning. The human Erk1 protein kinase has been shown to be widely expressed as a 44-kDa protein in many tissues. The human Erk2 protein kinase is a 41-kDa protein that is expressed ubiquitously. In contrast, a human Erk3-related protein kinase has been found to be expressed at a high level only in heart muscle and brain. The loci of these MAP kinase genes are widely distributed within the human genome: erk2 at 22q11.2; erk1 at 16p11.2; and ek3-related at 18q12-21. In the yeast Saccharomyces cerevisiae, five MAP kinase gene homologs have been described: smkl, mpk1, hog1, fus3, and kss1. Together, these kinases are a more diverse group than the human erks that have been identified. Thus the erks are likely to represent only one subgroup of a larger human MAP kinase gene family. A candidate for this extended family of MAP kinases is the c-Jun NH2-terminal kinase (Jnk), which binds to and phosphorylates the transcription factor c-Jun at

the activating sites Ser-63 and Ser-73. Evidence is presented here to demonstrate that Jnk is a distant relative of the MAP kinase group that is activated by dual phosphorylation at Tyr and Thr.

PMID: 8607977 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1995 May 19;270(20):11757-60.

Direct interaction between Ras and the kinase domain of mitogen-activated protein kinase kinase kinase (MEKK1).

Russell M, **Lange-Carter CA**, **Johnson GL**.

Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206, USA.

Mitogen-activated protein kinase kinase kinase (MEKK1) is a serine-threonine kinase that regulates sequential protein kinase pathways involving stress-activated protein kinases and mitogen-activated protein kinases. MEKK1 is activated in response to growth factor stimulation of cells and by expression of activated Ras. We demonstrate that the kinase domain of MEKK1 (MEKKCOOH) binds to GST-RasV12 in a GTP-dependent manner. Purified bacterially expressed MEKKCOOH binds to GST-RasV12(GTP gamma S) (GTP gamma S is guanosine 5'-3-O-(thio)triphosphate), demonstrating a direct interaction of the two proteins. A Ras effector domain peptide blocks the binding of MEKKCOOH to GST-RasV12(GTP gamma S). MEKKCOOH complexed with GST-RasV12(GTP gamma S) is capable of phosphorylating MEK1. These findings indicate that MEKK1 directly binds Ras.GTP. Thus, Ras interacts with protein kinases of both the Raf and MEKK families.

PMID: 7744823 [PubMed - indexed for MEDLINE]

1: Cell. 1992 Mar 20;68(6):1031-40.

Ras is essential for nerve growth factor- and phorbol ester-induced tyrosine phosphorylation of MAP kinases.

Thomas SM, **DeMarco M**, **D'Arcangelo G**, **Halegoua S**, **Brugge JS**.

Howard Hughes Medical Institute, Department of Microbiology, University of Pennsylvania, Philadelphia 19104.

Treatment of PC12 cells with nerve growth factor (NGF) induces a rapid increase in tyrosine phosphorylation of multiple cellular proteins. Expression of a dominant inhibitory Ras mutant specifically blocked NGF- and TPA-induced tyrosine phosphorylation of two proteins of approximately 42 and 44 kd. Conversely, expression of an oncogenic variant of Ras induced tyrosine phosphorylation of the same 42 and 44 kd proteins. The 44 kd protein was immunoprecipitated with an antibody directed against extracellular signal-regulated kinase 1/mitogen-activated protein kinase (MAPK) and the 42 kd protein comigrated with a 42 kd MAPK, indicating that at least one and probably both Ras-regulated phosphoproteins are MAPKs. In addition, MAPK activation, as measured by in vitro phosphorylation of myelin basic protein, was also regulated by Ras. Ras was not required for NGF-induced activation of Trk or tyrosine phosphorylation of PLC-gamma 1. Thus, NGF-induced tyrosine phosphorylation occurs both prior to and following Ras action, and Ras plays a critical role in the NGF- and TPA-induced tyrosine phosphorylation of MAPKs.

PMID: 1312392 [PubMed - indexed for MEDLINE]

1: Cancer Res. 1988 Jun 15;48(12):3470-7.

Transformation of normal homologous cells by a spontaneously activated Ha-ras oncogene.

Schwartz SA, **Shuler CF**, **Freebeck P**.

Department of Pathology, Chicago Medical School, Illinois 60064.

Several tumor-derived oncogenes have been shown to independently act as complete carcinogens following transfection into target cells from established tissue culture lines. However, the number and types of oncogenes required to transform primary cultures of normal mammalian cells is unclear. To clarify this issue in a simplified model system, we transfected genomic DNA from a naturally occurring rat tumor into NIH/3T3 cells as well as into early passage rat embryo fibroblasts. The 3T3 cells were transformed with high efficiency to malignant phenotypes; the rat embryo cells were transformed at lower frequencies following cotransfection with a selectable neomycin resistance marker and treatment with Geneticin (G418). The transformed rat cells had cancerous phenotypes as determined by in vitro, cytogenetic, and in vivo criteria. Moreover, the transformed mouse and rat cells contained new tumor DNA-derived nucleotide sequences homologous to the activated human Ha-ras oncogene. Elevated levels of Ha-ras-specific mRNA, as well as enhanced expression of the Mr 21,000 oncogene product, were detected in the transformed cells. Therefore, under well-defined experimental conditions, a spontaneously activated Ha-ras oncogene from a naturally occurring tumor was able to independently transform normal, homologous cells to a malignant phenotype.

PMID: 3285996 [PubMed - indexed for MEDLINE]

1: J Cell Physiol. 1982 May;111(2):140-8.

Links

Effects of 12-0-Tetradecanoylphorbol-13-acetate (TPA) on rat pheochromocytoma (PC12) cells: interactions with epidermal growth factor and nerve growth factor.

End D, **Tolson N**, **Yu MY**, **Guroff G**.

The phorbol ester tumor promotor 12-0-tetradecanoylphorbol-13-acetate (TPA) specifically inhibited the binding of radioiodinated epidermal growth factor (125I-EGF) to rat pheochromocytoma (PC12) cells in a noncompetitive fashion with an apparent Ki of 11-26 nM. Both TPA and EGF elicited similar biological responses in PC12 cells including enhanced incorporation of 3H-choline and 32 P-orthophosphate into macromolecules, induction of ornithine decarboxylase, and stimulation of the phosphorylation of a 30,000 MW nonhistone, chromosome-associated protein. These effects were also elicited by nerve growth fact (NGF) which, in contrast to the former agents, is a differentiating stimulus for PC12 cells. The effects of TPA were additive or more than additive to the effects of NGF and EGF. When PC12 cells were induced to differentiate by treatment with NGF for 72 hours, the binding of 125I-EGF and responses to EGF were reduced by approximately 70%. The response of PC12 cells to the tumor promoter TPA was unaffected by treatment with NGF. Thus, the qualitatively similar effects of TPA and EGF seemed to be mediated through separate receptor systems with only the EGF receptor system reduced by NGF treatment.

PMID: 6979547 [PubMed - indexed for MEDLINE]

1: Oncogene. 1994 Nov;9(11):3213-8.

Links

Signalling from TPA to MAP kinase requires protein kinase C, raf and MEK: reconstitution of the signalling pathway in vitro.

Marquardt B, **Frith D**, **Stabel S**.

Max-Delbruck-Laboratorium in der Max-Planck-Gesellschaft, Koln, Germany.

The phorbol ester PMA/TPA (phorbol 12-myristate 13-acetate) is a potent tumor promoter which mimics distinct intracellular signalling events triggered by activated growth factor receptors, e.g. the activation of MAP kinases. The largest known family of TPA-binding proteins comprise members of the protein kinase C (PKC) family although other TPA-binding proteins outside the PKC family have recently been identified. In this report we addressed the mechanism and the pathway by which TPA induces the activation of MAPkinases. Using recombinant proteins and in vitro phosphorylation reactions we identified the components in the signal transduction pathway from TPA to MAPkinase and we show that the activation of MAPkinase by TPA requires the presence of protein kinase C, c-raf and the MAPkinase activator MEK. We also find that the activation of raf autophosphorylation in vitro correlates with the ability of Raf to signal to MAPkinase. Thus the activation of Raf by PKC apparently can trigger the same signalling pathway as oncogenic Raf or Raf activation by ras in combination with tyrosine phosphorylation.

PMID: 7936644 [PubMed - indexed for MEDLINE]

1: Science. 1994 Sep 2;265(5177):1458-61.

Ras-dependent growth factor regulation of MEK kinase in PC12 cells.

Lange-Carter CA, **Johnson GL**.

Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

Mitogen-activated protein kinases (MAPKs) are rapidly activated in response to stimulation of diverse receptor types. MAPKs are positively regulated by phosphorylation on threonine and tyrosine by MAP kinase or extracellular signal-regulated kinase (ERK) kinases (MEKs). MEK kinase (MEKK) is part of a family of serine-threonine protein kinases that phosphorylate and activate MEKs independently of Raf. MEKK was rapidly and persistently activated in response to stimulation of resting PC12 cells with epidermal growth factor (EGF). Nerve growth factor (NGF) and 12-O-tetradecanoylphorbol-13-acetate (TPA) also activated MEKK, although to a lesser degree than did EGF. Activation of MEKK and B-Raf in response to EGF was inhibited by expression of dominant negative N17Ras. Expression of oncogenic Ras resulted in activation of MEKK. Stimulation of synthesis of cyclic adenosine 3',5'-monophosphate abolished activation of MEKK and B-Raf by EGF, NGF, and TPA. Thus, Ras simultaneously controls the activation of members of the Raf and MEKK families of protein kinases.

PMID: 8073291 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1998 Mar 20;273(12):7141-7.

Caspase-dependent cleavage of signaling proteins during apoptosis. A turn-off mechanism for anti-apoptotic signals.

Widmann C, Gibson S, Johnson GL.

Program in Molecular Signal Transduction, National Jewish Medical and Research Center, Denver, Colorado 80206, USA.

Caspases are activated during apoptosis and cleave specific proteins, resulting in the irreversible commitment to cell death. The signal transduction proteins MEKK1, p21-activated kinase 2, and focal adhesion kinase are caspase substrates that contribute to the cell death response when cleaved. Thirty additional signaling proteins were screened for their ability to be cleaved during apoptosis. Twenty-two of these proteins were not affected in Jurkat cells stimulated to undergo apoptosis by Fas ligation, exposure to ultraviolet-C or incubation with etoposide. Ras GTPase-activating protein was found to be a caspase substrate whose cleavage followed the same time course as that for activation of caspase activity and the cleavage of MEKK1 and focal adhesion kinase. Four additional proteins, Cbl, Cbl-b, Raf-1, and Akt-1, were cleaved later in the apoptotic response. These signaling proteins were similarly cleaved in U937 cells undergoing apoptosis. Cleavage of the proteins was blocked by caspase inhibitors in Jurkat cells or in U937 cells expressing BclxL, demonstrating that the cleavage was dependent on caspase activation. Cleavage of Raf-1 and Akt correlated with the loss of extracellular signal-regulated kinase and Akt activities in apoptotic cells. Neither c-Jun N-terminal kinase nor p38 mitogen-activated protein kinase was cleaved in cells undergoing apoptosis, and the activation of the c-Jun N-terminal kinase and p38 mitogen-activated protein kinase pathways was not compromised in apoptotic cells. These results indicate that caspase-dependent cleavage of specific proteins induces the turn off of survival pathways, such as the extracellular signal-regulated kinase and phosphatidylinositol-3 kinase/Akt pathways, that could otherwise interfere with the apoptotic response.

PMID: 9507028 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 2002 Jun 21;277(25):23065-75. Epub 2002 Apr 8.

c-Raf/MEK/ERK pathway controls protein kinase C-mediated p70S6K activation in adult cardiac muscle cells.

Iijima Y, Laser M, Shiraishi H, Willey CD, Sundaravadivel B, Xu L, McDermott PJ, Kuppuswamy D.

Cardiology Division of the Department of Medicine, Gazes Cardiac Research Institute, Medical University of South Carolina, Charleston, South Carolina 29425-2221, USA.

p70S6 kinase (S6K1) plays a pivotal role in hypertrophic cardiac growth via ribosomal biogenesis. In pressure-overloaded myocardium, we show S6K1 activation accompanied by activation of protein kinase C (PKC), c-Raf, and mitogen-activated protein kinases (MAPKs). To explore the importance of the c-Raf/MAPK kinase (MEK)/MAPK pathway, we stimulated adult feline cardiomyocytes with 12-O-tetradecanoylphorbol-13-acetate (TPA), insulin, or forskolin to activate PKC, phosphatidylinositol-3-OH kinase, or protein kinase A (PKA), respectively. These treatments resulted in S6K1 activation with Thr-389 phosphorylation as well as mammalian target of rapamycin (mTOR) and S6 protein phosphorylation. Thr-421/Ser-424 phosphorylation of S6K1 was observed predominantly in TPA-treated cells. Dominant negative c-Raf expression or a MEK1/2 inhibitor (U0126) treatment showed a profound blocking effect only on the TPA-stimulated phosphorylation of S6K1 and mTOR. Whereas p38 MAPK inhibitors exhibited only partial effect, MAPK-phosphatase-3 expression significantly blocked the TPA-stimulated S6K1 and mTOR phosphorylation. Inhibition of mTOR with rapamycin blocked the Thr-389 but not the Thr-421/Ser-424 phosphorylation of S6K1. Therefore, during PKC activation, the c-Raf/MEK/extracellular signal-regulated kinase-1/2 (ERK1/2) pathway mediates both the Thr-421/Ser-424 and the Thr-389 phosphorylation in an mTOR-independent and -dependent manner, respectively. Together, our in vivo and in vitro studies indicate that the PKC/c-Raf/MEK/ERK pathway plays a major role in the S6K1 activation in hypertrophic cardiac growth.

PMID: 11940578 [PubMed - indexed for MEDLINE]

Volume 230, Issue 2,
Pages 248-259 (18 December 2005)

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Involvement of Bcl-2 family, cytochrome *c* and caspase 3 in induction of apoptosis by beauvericin in human non-small cell lung cancer cells

Hen-I. Lin[ab], Yih-Jing Lee[a], Bing-Fang Chen[a], Meng-Chao Tsai[a], Jen-Lin Lu[a], Cheng-Jen Chou[c], Guey-Mei Jow[a]

Received 30 November 2004; received in revised form 27 December 2004; accepted 29 December 2004

ABSTRACT
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Abstract

Beauvericin (BEA), a cyclic hexadepsipeptide from *Codyceps cicadae*, possesses anti-convulsion, anti-arrhythmia, sedation, and anti-tumor activities. It has been reported that BEA induces apoptosis in several cancer cell lines. However, the molecular mechanism underlying the BEA-induced apoptotic process is not yet clearly understood. In the present study, the intracellular signaling pathways of BEA-induced apoptosis in human non-small cell lung cancer (NSCLC) A549 cells were investigated using morphological analysis and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) technique. In this study, BEA-induced apoptosis in human NSCLC A549 cells demonstrated a BEA concentration- and treatment time-dependent manner. This BEA-induced apoptosis in human NSCLC A549 cells was also accompanied by the up-regulation of Bax, Bak, and p-Bad and down-regulation of p-Bcl-2, but no effect on the levels of Bcl-X_L or Bad proteins. Moreover, the BEA treatment resulted in a significant reduction of mitochondrial membrane potential, increase in the release of mitochondrial cytochrome *c* (cyt *c*), and activation of caspase 3. Furthermore, treatment with caspase 3 inhibitor (z-DEVD-fmk) was capable to prevent the BEA-induced caspase 3 activity and cell death. These results clearly demonstrate that the induction of apoptosis by BEA involves multiple cellular/molecular pathways and strongly suggest that pro- and anti-apoptotic Bcl-2 family proteins, mitochondrial membrane potential, mitochondrial cyt *c*, and caspase 3, they all participate in BEA-induced apoptotic process in human NSCLC A549 cells.

Keywords: Beauvericin, Apoptosis, Bcl-2, Cytochrome *c*, Caspase 3

1: Cancer Lett. 2004 Dec 28;216(2):165-73.

Beauvericin induces cytotoxic effects in human acute lymphoblastic leukemia cells through cytochrome c release, caspase 3 activation: the causative role of calcium.

Jow GM, **Chou CJ**, **Chen BF**, **Tsai JH**.

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Beauvericin (BEA), a cyclic hexadepsipeptide, induces cell death in human leukemia cells (CCRF-CEM) and the process of BEA-induced cell death has been speculated to undergo an apoptotic pathway. In the present study, several well-characterized factors, known to play important roles in apoptotic pathway, were investigated in BEA-induced CCRF-CEM cell death. CCRF-CEM cells were treated with BEA at concentrations from 1 to 10 microM for up to 24 h. The incidence of nuclear fragmentation and apoptotic body formation in the cells, cytosolic caspase-3 activity, mitochondrial membrane potential, and release of cytochrome c (Cyt c) from mitochondria in BEA-treated cells were determined and compared with that in untreated cells. Moreover, to investigate the role of intracellular Ca++ in this cell death process, CCRF-CEM cells were primed with 3 microM of BAPTA/AM, a Ca++ chelator, to exclude intracellular Ca++ prior to the BEA treatment. The data revealed that BEA-induced cell death in CCRF-CEM cells exhibited a dose- and time-dependent manner. The incidence of nuclear fragmentation and apoptotic body formation was significantly increased in CCRF-CEM cells treated with BEA at concentrations of 1 microM or greater. Increase of cytosolic caspase-3 activity was also observed in BEA-treated cells with a dose-dependent manner. In addition, increased release of Cyt c from mitochondria was also observed in the cells treated with 10 microM BEA in a time-dependent pattern. The BAPTA/AM pretreatment partially blocked BEA-induced cell death in CCRF-CEM cells, indicating that intracellular Ca++ plays an important role, maybe as a mediator in cell death signaling, in this cell death pathway. The results support the notion that BEA-induced cell death in CCRF-CEM cells likely undergo through an apoptotic pathway on the basis of increase of release of Cyt c from mitochondria, increase of caspase-3 activity, and some observed typical apoptotic

cellular changes in morphology.

PMID: 15533592 [PubMed - indexed for MEDLINE]

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Volume 216, Issue 2,
Pages 165-173 (28 December 2004)

previous 4 of 16 next

Beauvericin induces cytotoxic effects in human acute lymphoblastic leukemia cells through cytochrome *c* release, caspase 3 activation: the causative role of calcium

Guey-Mei Jow[a], Cheng-Jen Chou[b], Bing-Fang Chen[a], Jia-Huei Tsai[a]

Received 18 February 2004; received in revised form 1 June 2004; accepted 2 June 2004

Abstract

Beauvericin (BEA), a cyclic hexadepsipeptide, induces cell death in human leukemia cells (CCRF-CEM) and the process of BEA-induced cell death has been speculated to undergo an apoptotic pathway. In the present study, several well-characterized factors, known to play important roles in apoptotic pathway, were investigated in BEA-induced CCRF-CEM cell death. CCRF-CEM cells were treated with BEA at concentrations from 1 to 10μM for up to 24h. The incidence of nuclear fragmentation and apoptotic body formation in the cells, cytosolic caspase-3 activity, mitochondrial membrane potential, and release of cytochrome *c* (Cyt *c*) from mitochondria in BEA-treated cells were determined and compared with that in untreated cells. Moreover, to investigate the role of intracellular Ca^{++} in this cell death process, CCRF-CEM cells were primed with 3μM of BAPTA/AM, a Ca^{++} chelator, to exclude intracellular Ca^{++} prior to the BEA treatment. The data revealed that BEA-induced cell death in CCRF-CEM cells exhibited a dose- and time-dependent manner. The incidence of nuclear fragmentation and apoptotic body formation was significantly increased in CCRF-CEM cells treated with BEA at concentrations of 1μM or greater. Increase of cytosolic caspase-3 activity was also observed in BEA-treated cells with a dose-dependent manner. In addition, increased release of Cyt *c* from mitochondria was also observed in the cells treated with 10μM BEA in a time-dependent pattern. The BAPTA/AM pretreatment partially blocked BEA-induced cell death in CCRF-CEM cells, indicating that intracellular Ca^{++} plays an important role, maybe as a mediator in cell death signaling, in this cell death pathway. The results support the notion that BEA-induced cell death in CCRF-CEM cells likely undergo through an apoptotic pathway on the basis of increase of release of Cyt *c* from mitochondria, increase of caspase-3activity, and some observed typical apoptotic cellular

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PII: S0304-3835(04)00455-0

doi:10.1016/j.canlet.2004.06.005

1: Br J Cancer. 1995 Mar;71(3):556-61.

Constitutive expression of the c-H-ras oncogene inhibits doxorubicin-induced apoptosis and promotes cell survival in a rhabdomyosarcoma cell line.

Nooter K, Boersma AW, Oostrum RG, Burger H, Jochemsen AG, Stoter G.

Department of Medical Oncology, University Hospital Rotterdam, The Netherlands.

Drugs used in anti-cancer chemotherapy are thought to exert their cytotoxic action by induction of apoptosis. Genes have been identified which can mediate or modulate this drug-induced apoptosis, among which are c-myc, p53 and bcl-2. Since expression of oncogenic ras genes is a frequent observation in human cancer, we investigated the effects of the c-H-ras oncogene on anti-cancer drug-induced apoptosis. Apoptosis induced by a 2 h doxorubicin exposure was measured by in situ nick translation and flow cytometry in a rat cell line (R2T24) stably transfected with the c-H-ras oncogene and in a control cell line (R2NEO) transfected only with the antibiotic resistance gene neo. Both cell lines (R2T24 and R2NEO) had nearly identical growth characteristics, including cell doubling time, distribution over the cell cycle phases and plating efficiency in soft agar. Doxorubicin exposure of the R2NEO cells led to massive induction of apoptosis. In contrast, R2T24 cells, expressing the c-H-ras oncogene, showed significantly less apoptosis after doxorubicin incubation. Doxorubicin induced approximately 3- to 5-fold less cytotoxicity in the R2T24 cells than in the R2NEO cells, as determined by clonogenic assay in soft agar. No difference was observed in intracellular doxorubicin accumulation between the two cell lines, indicating that the classical, P-glycoprotein-mediated multidrug resistance phenotype is not involved in the observed differences in drug sensitivity. In conclusion, our data show that constitutive expression of the c-H-ras oncogene suppresses doxorubicin-induced apoptosis and promotes cell survival, suggesting that human tumours with ras oncogene expression might be less susceptible to doxorubicin treatment.

PMID: 7880739 [PubMed - indexed for MEDLINE]

1: Biotechnol Lett. 2006 Sep 23; [Epub ahead of print]

Cloning and characterization of a novel small monomeric GTPase, RasL10B, with tumor suppressor potential.

Zou H, **Hu L**, **Li J**, **Zhan S**, **Cao K**.

State Key Laboratory of Genetic Engineering, Department of Biochemistry, School of Life Sciences, Fudan University, Shanghai, 200433, PR China, kmcao@fudan.edu.cn.

Ras proteins are members of the superfamily of small GTPase. A novel human Ras-like transcript, termed RasL10B, was isolated from human blood cell cDNA library. RasL10B gene contains four exons and three introns, which encodes a 203 amino acid protein with a molecular mass of about 23.2 kDa. RT-PCR analysis showed that RasL10B is expressed extensively in human tissues. Subcellular location analysis of GFP-RasL10B fusion protein revealed that RasL10B was distributed to the cytoplasm of COS7 cells. In addition, RasL10B was expressed in E. coli Rosette (DE3) and purified to a homogenicity by Ni-NTA affinity chromatography. Finally, the mRNA levels of RasL10B were down-regulated in all human breast cancer cell lines we tested. In summary, RasL10B is a new member of Ras superfamily with tumor suppressor potential.

PMID: 17028781 [PubMed - as supplied by publisher]

1: J Virol. 1982 Nov;44(2):509-19.

Comparative biochemical properties of p21 ras molecules coded for by viral and cellular ras genes.

Papageorge A, Lowy D, Scolnick EM.

In earlier studies, we molecularly cloned a normal cellular gene, c-rasH-1, homologous to the v-ras oncogene of Harvey murine sarcoma virus (v-rasH). By ligating a type c retroviral promotor to c-rasH-1, we could transform NIH 3T3 cells with the c-rasH-1 gene. The transformed cells contained high levels of a p21 protein coded for by the c-rasH-1 gene. In the current studies, we have purified extensively both v-rasH p21 and c-rasH p21 and compared the in vivo and in vitro biochemical properties of both these p21 molecules. The p21 proteins coded for by v-rasH and c-rasH-1 shared certain properties: each protein was synthesized as a precursor protein which subsequently became bound to the inner surface of the plasma membrane; each protein was associated with guanine nucleotide-binding activity, a property which copurified with p21 molecules on a high-pressure liquid chromatography molecular sizing column. In some other properties, the v-rasH and c-rasH p21 proteins differed. In vivo, approximately 20 to 30% of v-rasH p21 molecules were in the form of phosphothreonine-containing pp21 molecules, whereas in vivo only a minute fraction of c-rasH-1 p21 contained phosphate, and this phosphate was found on a serine residue. v-rasH pp21 molecules with an authentic phosphothreonine peptide could be synthesized in vitro in an autophosphorylation reaction in which the gamma phosphate of GTP was transferred to v-rasH p21. No autophosphorylating activity was associated with purified c-rasH-1 p21 in vitro. The results indicate a major qualitative difference between the p21 proteins coded for by v-rasH and c-rasH-1. The p21 coded for by a mouse-derived oncogenic virus, BALB murine sarcoma virus, resembled the p21 coded for by c-rasH-1 in that it bound guanine nucleotides but did not label appreciably with 32Pi. The forms of p21 coded for by other members of the ras gene family were compared, and the results indicate that the guanine nucleotide-binding activity is common to p21 molecules coded for by all known members of the ras gene family.

PMID: 6292515 [PubMed - indexed for MEDLINE]

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1: Cancer Surv. 1986;5(2):275-89.

Links

The ras gene family.

Lowy DR, **Willumsen BM**.

Members of the ras multigene family have been found in virtually all eukaryotes, from yeast to mammals. ras is required for normal cell growth in the yeast Saccharomyces cerevisiae and in at least some mammalian cells. These genes induce tumorigenic transformation of established NIH 3T3 cells by increased expression of a normal ras gene, certain point mutations or amino acid deletion. In tumours, point mutation appears to be the most common mechanism of activation. The ras proteins are found at the plasma membrane, bind guanine nucleotides GDP and GTP and possess a GTPase activity. At least some ras proteins that have been activated by single amino acid substitutions possess a GTPase activity that is lower than that of the normal version. These results are consistent with the hypothesis that ras protein stimulates its putative target(s) when GTP is bound to it, as is true for the G regulatory proteins or elongation factor Tu. In Saccharomyces cerevisiae, ras has been shown to stimulate adenylate cyclase. However, there does not appear to be a direct interaction between ras and adenylate cyclase in mammalian cells.

PMID: 3096566 [PubMed - indexed for MEDLINE]

1: Nature. 1984 Aug 23-29;310(5979):644-9.

Links

Comparative biochemical properties of normal and activated human ras p21 protein.

McGrath JP, Capon DJ, Goeddel DV, Levinson AD.

Human Ha-ras1 cDNAs encoding normal and activated p21 polypeptides have been efficiently expressed in Escherichia coli and the biochemical activities associated with each polypeptide compared. In addition to the guanine nucleotide binding activity, normal p21 displays a GTPase activity which is selectively impaired by a mutation which activates its oncogenic potential.

PMID: 6147754 [PubMed - indexed for MEDLINE]

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Biochemical characterization of polypeptides encoded by mutated human Ha-ras1 genes. [Mol Cell Biol. 1986]

Deletion mutants of Harvey ras p21 protein reveal the absolute requirement of at least two distant regions for GTP-binding and transforming activities. [EMBO J. 1986]

Expression of p21 proteins in Escherichia coli and stereochemistry of the nucleotide-binding [EMBO J. 1986]

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L15 ANSWER 73 OF 93 MEDLINE on STN
ACCESSION NUMBER: 90072941 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2686537
TITLE: ras proteins: biological effects and biochemical targets (review).
AUTHOR: Bar-Sagi D
CORPORATE SOURCE: Cold Spring Harbor Laboratory, New York 11724.
CONTRACT NUMBER: CA46370 (NCI)
SOURCE: Anticancer research, (1989 Sep-Oct) Vol. 9, No. 5, pp. 1427-37. Ref: 89
Journal code: 8102988. ISSN: 0250-7005.
PUB. COUNTRY: Greece
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199001
ENTRY DATE: Entered STN: 28 Mar 1990
Last Updated on STN: 3 Mar 2000
Entered Medline: 3 Jan 1990

AB Ras genes are an ubiquitous eukaryotic gene family. Since their discovery as the cellular homologues of the transforming genes of Harvey and Kirsten retroviruses, ras genes have been presumed to play a role in growth control, mainly because of their potential to induce uncontrolled cell proliferation. This notion is strongly supported by recent evidence indicating that ras mutations may be causative or closely linked to the onset of some types of human tumors. However, the mechanism of action of ras proteins in mammalian cells is poorly understood. Using the microinjection technique as a biological assay for ras proteins, it has been possible to address several important questions concerning cellular and biochemical aspects of ras function. When introduced into living cells by microinjection, purified ras proteins can induce cell proliferation, neuronal differentiation, oocyte maturation, and exocytotic degranulation. On the biochemical level, microinjection studies indicated that ras proteins can induce specific alterations in phospholipid metabolism.

Science 23 October 1987:
Vol. 238. no. 4826, pp. 533 - 536
DOI: 10.1126/science.2821623

ARTICLES

Science, Vol 238, Issue 4826, 533-536

ARTICLES

Rapid stimulation of diacylglycerol production in Xenopus oocytes by microinjection of H-ras p21

JC Lacal, P de la Pena, J Moscat, P Garcia-Barreno, PS Anderson, and SA Aaronson

Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892.

The p21 products of ras proto-oncogenes are thought to be important components in pathways regulating normal cell proliferation and differentiation. These proteins acquire transforming properties as a result of activating lesions that convert ras genes to oncogenes in a wide array of malignancies. In Xenopus laevis oocytes, microinjection of transforming ras p21 is a potent inducer of maturation, whereas microinjection of a monoclonal antibody to ras p21 inhibits normal maturation induced by hormones. The phosphoinositide pathway is a ubiquitous system that appears to play a key role in diverse cellular functions. By use of the Xenopus oocyte system, it was possible to quantitate the effects of ras p21 microinjection on individual components of the phosphoinositide pathway. Within 20 minutes of microinjection, levels of phosphatidylinositol 4,5-bisphosphate, inositol 1-phosphate, and inositol bisphosphate increased 1.5- to 2-fold. The most striking effects were on diacylglycerol, which increased 5-fold under the same conditions. In contrast, the normal ras p21 protein induced no detectable alteration in any of the metabolites analyzed. The earliest effects of the transforming p21 on phosphoinositol turnover were observable within 2 minutes, implying a very rapid effect of ras p21 on the enzymes involved in phospholipid metabolism.

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Ronen (2005)
Cancer Res. **65**, 3356-3363
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Protein Kinase C and Mitogen-Activated Protein Kinase Cascade in Mouse Cumulus Cells: Cross Talk and Effect on Meiotic Resumption of Oocyte.
H.-Y. Fan, L.-J. Huo, D.-Y. Chen, H. Schatten, and Q.-Y. Sun (2004)
Biol Reprod **70**, 1178-1187
Abstract » **Full Text »** **PDF »**

Sphingomyelin Synthase, a Potential Regulator of Intracellular Levels of Ceramide and Diacylglycerol during SV40 Transformation. DOES SPHINGOMYELIN SYNTHASE ACCOUNT FOR THE PUTATIVE PHOSPHATIDYLCHOLINE-SPECIFIC PHOSPHOLIPASE C?.
C. Luberto and Y. A. Hannun (1998)
J. Biol. Chem. **273**, 14550-14559
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Mapping and Functional Role of Phosphorylation Sites in the Thyroid Transcription Factor-1 (TTF-1).
M. Zannini, A. Acebron, M. De Felice, M. I. Arnone, J. Martin-Pérez, P. Santisteban, and R. Di Lauro (1996)
J. Biol. Chem. **271**, 2249-2254
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The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product.
C. Crews, A Alessandrini, and R. Erikson (1992)
Science **258**, 478-480
Abstract » **PDF »**

10

15 ANSWER 78 OF 93 MEDLINE on STN DUPLICATE 28
ACCESSION NUMBER: 87172752 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3550423
TITLE: Biochemical and biological properties of the human N-ras p21 protein.
AUTHOR: Trahey M; Milley R J; Cole G E; Innis M; Paterson H; Marshall C J; Hall A; McCormick F
SOURCE: Molecular and cellular biology, (1987 Jan) Vol. 7, No. 1, pp. 541-4.
Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198704
ENTRY DATE: Entered STN: 3 Mar 1990
Last Updated on STN: 3 Mar 2000
Entered Medline: 27 Apr 1987

AB We characterized the normal (Gly-12) and two mutant (Asp-12 and Val-12) forms of human N-ras proteins produced by Escherichia coli. No significant differences were found between normal and mutant p21 proteins in their affinities for GTP or GDP. Examination of GTPase activities revealed significant differences between the mutant p21s: the Val-12 mutant retained 12% of wild-type GTPase activity, whereas the Asp-12 mutant retained 43%. Both mutant proteins, however, were equally potent in causing morphological transformation and increased cell motility after their microinjection into quiescent NIH 3T3 cells. This lack of correlation between transforming potency and GTPase activity or guanine nucleotide binding suggests that position 12 mutations affect other aspects of p21 function.

QH573.C38 Page 1 of 1
adonis QH573.C38 mmmdlm

Cell

1: Cell. 1984 Aug;38(1):109-17.

Microinjection of the oncogene form of the human H-ras (T-24) protein results in rapid proliferation of quiescent cells.

Feramisco JR, **Gross M**, **Kamata T**, **Rosenberg M**, **Sweet RW**.

Using an E. coli expression-vector system we have efficiently produced, purified, and characterized the full-length, nonfused, protooncogenic and oncogenic (T-24) forms of the human H-ras gene product. These purified ras proteins have been introduced by microinjection into a variety of somatic cells in an effort to examine their function. Within several hours after injection of the oncogenic form of the human H-ras protein into quiescent cells, we observe dramatic morphological changes followed by transient proliferation of the cells. In contrast, microinjection of the normal, protooncogenic form of the ras protein at the same level appears to have only little effect on the cells. Additional experiments indicate that the effect of the ras protein requires entry into the cells, is temporary, is inhibited by cycloheximide or actinomycin D, and is seen only in established cell lines. This experimental approach demonstrates that the bacterially derived and purified human H-ras proteins retain their ability to function when put back into mammalian cells and furthermore, provides a novel assay for transformation induced in established cells by the human H-ras oncogene protein.

PMID: 6380758 [PubMed - indexed for MEDLINE]

cell

Cell

Volume 44, Issue 4 , 28 February 1986, Pages 609-617

doi:10.1016/0092-8674(86)90270-9 Cite or Link Using DOI

Article

Ras p21 proteins with high or low GTPase activity can efficiently transform NIH/3T3 cells

Juan Carlos Lacal, Shiv K. Srivastava, Paul S. Anderson and Stuart A. Aaronson

Laboratory of Cellular and Molecular Biology National Cancer Institute Building 37, Room 1E24, Bethesda, Maryland 20892, USA

Received 27 August 1985; Revised 4 November 1985. Available online 7 May 2004.

Abstract

We sought to determine whether decreased in vitro GTPase activity is uniformly associated with *ras* p21 mutants possessing efficient transforming properties. Normal H-*ras* p21-[Gly^{12}-Ala^{59}] as well as an H-*ras* p21-[Gly^{12}-Thr^{59}] mutant exhibited in vitro GTPase activities at least fivefold higher than either H-*ras* p21-[Lys^{12}-Ala^{59}] or H-*ras* p21-[Arg^{12}-Thr^{59}] mutants. Microinjection of as much as 6×10^6 molecules/cell of bacterially expressed normal H-*ras* p21 induced no detectable alterations of NIH/3T3 cells. In contrast, inoculation of 4–5 $\times 10^5$ molecules/cell of each p21 mutant induced morphologic alterations and stimulated DNA synthesis. Moreover, the transforming activity of each mutant expressed in a eukaryotic vector was similar and at least 100-fold greater than that of the normal H-*ras* gene. These findings establish that activation of efficient transforming properties by *ras* p21 proteins can occur by mechanisms not involving reduced in vitro GTPase activity.

Cell
Volume 44, Issue 4 , 28 February 1986, Pages 609-617

Cell

Volume 38, Issue 1 , August 1984, Pages 109-117

doi:10.1016/0092-8674(84)90531-2 Cite or Link Using DOI

Article

Microinjection of the oncogene form of the human H-*ras* (t-24) protein results in rapid proliferation of quiescent cells

James R. Feramisco*, Mitchell Gross†, Tohru Kamata*, Martin Rosenberg† and Raymond W. Sweet†

* Cold Spring Harbor Laboratory Cold Spring, Harbor, New York 11724, USA
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Received 8 June 1984. Available online 29 April 2004.

Abstract

Using an E. coli expression-vector system we have efficiently produced, purified, and characterized the full-length, nonfused, protooncogenic and oncogenic (T-24) forms of the human H-*ras* gene product. These purified *ras* proteins have been introduced by microinjection into a variety of somatic cells in an effort to examine their function. Within several hours after injection of the oncogenic form of the human H-*ras* protein into quiescent cells, we observe dramatic morphological changes followed by transient proliferation of the cells. In contrast, microinjection of the normal, protooncogenic form of the *ras* protein at the same level appears to have only little effect on the cells. Additional experiments indicate that the effect of the ras protein requires entry into the cells, is temporary, is inhibited by cycloheximide or actinomycin D, and is seen only in established cell lines. This experimental approach demonstrates that the bacterially derived and purified human H-*ras* proteins retain

their ability to function when put back into mammalian cells and furthermore, provides a novel assay for transformation induced in established cells by the human H-*ras* oncogene protein.

Cell
Volume 38, Issue 1 , August 1984, Pages 109-117